Transplant Infectious Disease

WILEY

CLINICOPATHOLOGIC CONFERENCE OPEN ACCESS

Old Pathogens–New Patient Types: Infections in a CAR T-Cell Recipient. Could It Get Any More Complicated?

Monica Melchio[1,2] | Joshua A. Hill[3,4] | Maunank Shah[5,6] | Dionysios Neofytos[7] | Massimiliano Gambella[8] | Anna Maria Raiola[8] | Emanuele Delfino[2] | Elisa Balletto[2] | Emanuele Angelucci[8] | Matteo Bassetti[1,2] | Malgorzata Mikulska[1,2]

[1]Division of Infectious Diseases, Department of Health Sciences, University of Genova, Genova, Italy | [2]Division of Infectious Diseases, IRCCS Ospedale Policlinico San Martino, Genova, Italy | [3]Fred Hutchinson Cancer Center, Seattle, Washington, USA | [4]University of Washington, Seattle, Washington, USA | [5]Division of Infectious Diseases, School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA | [6]Department of Epidemiology, School of Public Health, Center for TB Research and Center for Clinical Global Health Education, Johns Hopkins University, Baltimore, Maryland, USA | [7]Division of Infectious Diseases, Transplant Unit, University Hospitals of Geneva, Geneva, Switzerland | [8]Division of Hematology and Cellular Therapy, IRCCS Ospedale Policlinico San Martino, Genova, Italy

Correspondence: Malgorzata Mikulska (m.mikulska@unige.it)

ABSTRACT

The case discussed involves a 41-year-old Italian man who was a candidate for chimeric antigen receptor T-cell therapy (CAR-T) for mediastinal diffuse large B-cell lymphoma. His CAR-T treatment was postponed several times due to prolonged relapsing COVID-19 and new onset of pulmonary *Mycobacterium tuberculosis* diseases. After 11 weeks of antimycobacterial treatment, CAR T-cell therapy was performed, but complicated by cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). Two months after CAR-T, the patient developed invasive pulmonary aspergillosis due to *A. fumigatus*. He was successfully treated with a 6-month course of antitubercular therapy and an 8-month course of antifungal therapy with isavuconazole. Lobectomy was performed due to episodes of severe hemoptysis. The challenging issues of diagnosis, choice, and management of treatments, including drug–drug interactions and length of therapy, are discussed.

1 | Initial Presentation

A 41-year-old male of Italian origin with no previous medical history, who worked in an office, with no history of international travel, developed fever and cough in early 2021 leading to the diagnosis of diffuse large B-cell lymphoma (DLBCL) with a large mediastinal mass (13 × 14 × 16 cm). The subsequent clinical history described in this paper is summarized in Figure 1. The full discussion is availabe online (Video 1).

In the following months, he underwent first-line chemotherapy (with standard rituximab containing DA-EPOCH scheme) at hematology department in the northern Italian region, resulting in partial remission. Thus, he underwent second-line chemotherapy (R-DHAOX regimen). PET scan performed at the end of treatment documented the persistence of residual disease while lung CT scan showed cavitation and subsequent consolidation of mediastinal mass, difficult to distinguish from pulmonary parenchyma (Figure 2).

Thus, 9 months after the diagnosis of lymphoma, the patient was considered a candidate for chimeric antigen receptor (CAR) T-cell therapy (Figure 1, time 0). Lymph apheresis was performed, followed by bridging with mediastinal radiotherapy.

While undergoing radiotherapy, the patient developed mild respiratory symptoms and tested positive for SARS-CoV-2 (Delta variant). He had previously received three doses of a COVID-19 mRNA vaccine, the last administered only two weeks prior to SARS-CoV-2 infection.

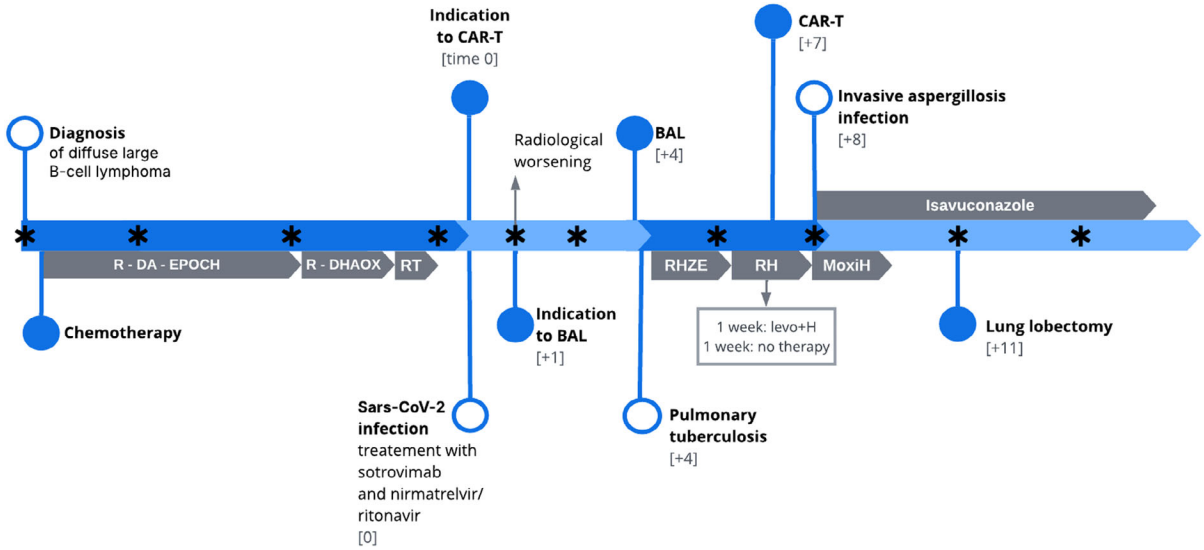

FIGURE 1 | Clinical timeline, shown in months from time 0, defined as the date of CAR-T treatment indication. Asterisks represent times when radiologic re-evaluation with CT or PET scan was performed. BAL, bronchoalveolar lavage; CAR-T, Chimeric Antigen Receptor T-cell; moxiH, moxifloxacin, isoniazid; R-DA-EPOCH, etoposide phosphate, prednisone, vincristine sulfate, cyclophosphamide, doxorubicin hydrochloride, and rituximab; R—DHAOX, rituximab, dexamethasone, high-dose cytarabine, and oxaliplatin; RHZE rifampicin, isoniazid, pyrazinamide, ethambutol; RH, rifampicin, isoniazid; RT, radiotherapy.

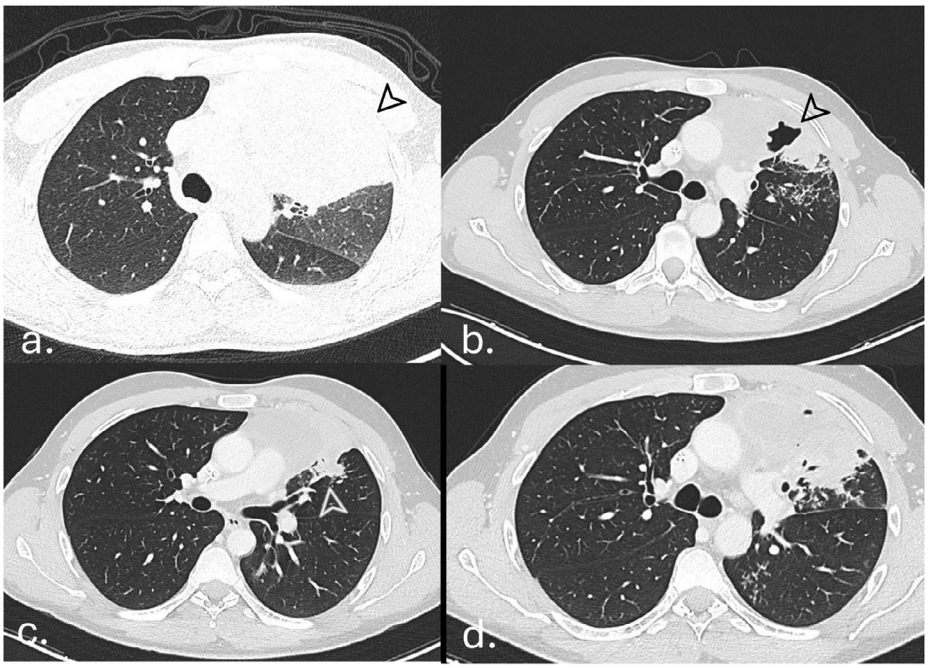

FIGURE 2 | CT scan showing the mediastinal mass of DLBCL before (a), during (b), and after first-line (c) and second-line (d) chemotherapy. The lesion was in the mediastinum, but as it was very close to the upper left lobe, on imaging it was not always very clear if the lung was involved or not.

2 | ID Problem No. 1: Sars-CoV-2

At that time, the patient was asymptomatic and was treated for COVID-19 in a hospital near his hometown with the current (end of year 2021) standard of care, that is, a monoclonal anti-Spike antibody (single dose of sotrovimab), and CAR-T was postponed. In the following months, he had relapsing mild upper respiratory tract symptoms, and he remained persistently positive by PCR for SARS-CoV-2.

3 | Question 1: How Long Should We Wait for CAR T-Cell Treatment After SARS-CoV-2 Infection?

JH: This clinical scenario lacks a definitive answer, which is common in practice. The following summarizes various guidelines on management of allogeneic hematopoietic cell transplant (HCT) recipients in the context of other respiratory viruses, which have evolved rapidly:

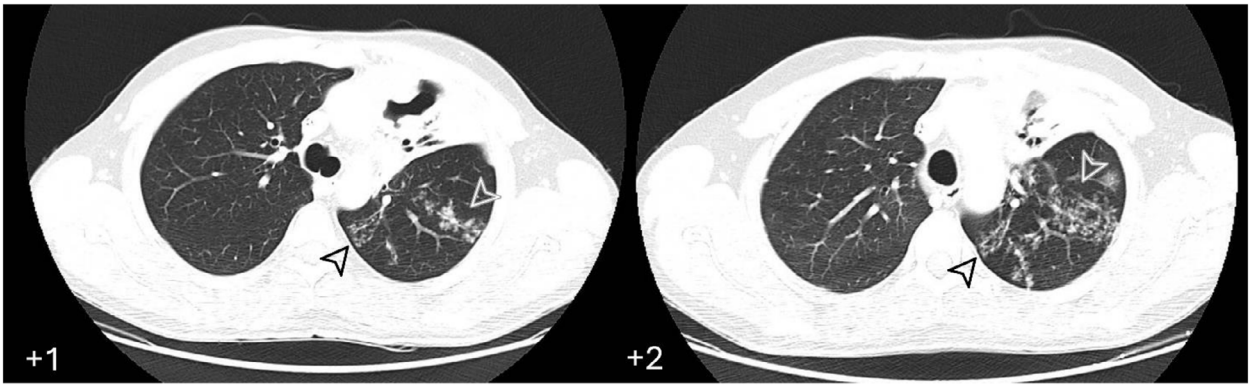

FIGURE 3 | At 1 (+1) and 2 (+2) months after COVID-19 diagnosis (concomitant with indication for CAR-T therapy, time 0), the CT scan did not show any typical signs of COVID-19 pneumonia. However, new lesions with a tree-in-bud shape (arrows), not present in previous scans, appeared and were confirmed at the following scan.

- European Guidelines EBMT (2021): deferral until resolution of infection is suggested [1].
- ASTCT Clinical Practice Guidelines (2022): for asymptomatic patients, a negative PCR result is not necessary; a waiting period of approximately 2 weeks after a positive test is suggested. For symptomatic patients, the recommendation is to wait until clinical improvement is observed. Even if the PCR remains positive after 2 to 3 weeks, proceeding to HCT/CAR-T may be considered [2].
- ECIL Guidelines (2023): deferral for up to 3 months is recommended [3]; the recent update (2024) a deferral until clinical and virological recovery is considered appropriate, depending on the severity of COVID-19 and vaccination status, but taking into consideration a clinical risk/benefit ratio assessment [4].

In this specific scenario, the patient had received three vaccine doses and sotrovimab; moreover outcomes in 2022 have improved significantly compared to the initial period: the mortality rate for patients undergoing CAR T-cell therapy or transplant during a COVID-19 infection is now 5%–10%, compared to the initial 30%–40% [5].

However, recent data still indicate that pretransplant respiratory viral infections involving the lower respiratory tract are associated with poorer outcomes in allogeneic HCT recipients [6]. Based on the above data, I would recommend waiting at least 2 weeks to monitor clinical improvement before proceeding, regardless of PCR results. This approach is particularly pertinent for CAR T-cell therapy recipients, who often have uncontrolled malignancy that precludes further delays.

4 | ID Problem No. 2: Prolonged COVID-19

After 1 month of persistent mild COVID-19, a new CT scan was performed to evaluate lung involvement in prolonged SARS-CoV-2 infection and the potential progression of lymphoma (Figure 3). CT scan did not show any typical sign of COVID-19 pneumonia. However, new lesions, not present in previous scan, with a tree-in-bud shape, appeared.

Considering the evolving radiological findings, a bronchoscopy with bronchoalveolar lavage (BAL) for diagnostic purpose was recommended. However, the procedure was postponed several times in the following 3 months due to the patient's SARS-CoV-2 positivity and intermittent mild clinical symptoms. The patient experienced alternating periods of SARS-CoV-2 swab positivity and negativity, making it difficult to discern whether this was due to intermittent viral shedding or a new Sars-CoV-2 infection, possibly due to the rapid spread of the Omicron variant, reported to affect increasing number of hematologic patients during those months, although sequencing was not performed [7]. The patient was treated with two 5-day courses of nirmatrelvir/ritonavir, with transient improvement in mild symptoms, but no negative result. His serology was negative for anti-S2 antibodies and anti-IgN antibodies, with anti S1 IgG antibody levels of 22 000 BAU/mL due to previous sotrovimab administration.

At that time, centers were unwilling to proceed with CAR T-cell therapy in a patient positive for Sars-CoV-2 and with mild respiratory symptoms, so the cellular treatment was postponed. Radiological monitoring was continued and it confirmed the evolution of lung involvement with tree-in-bud lesions, but no progression of lymphoma (Figure 3). After the indication to CAR-T was made, and during the Sars-CoV-2 infection, the patient was not undergoing active treatment for his lymphoma: chemotherapy and radiotherapy had been terminated 4 and 2 months earlier. For the management of his systemic symptoms possibly attributable to lymphoma (low-grade fever), he was receiving low-dose corticosteroids, in association with prophylaxis with trimethoprim/sulfamethoxazole and valacyclovir.

Laboratory testing documented a mild neutrophilia and a normal lymphocytes count (11 200 and 1550 mm^3, respectively), total IgG were 3.890 g/L (normal value 8–17) and total IgM were 0.042 g/L (normal value 0.4–2.3). Furthermore, the patient remained in overall good clinical condition, and despite the SARS-CoV-2 infection, he reported being able to work from home nearly every day. His symptoms were notably mild and he reported no sputum production.

In order to investigate the new pulmonary lesions and to increase sensitivity of diagnosis and enable testing for multiple pathogens, BAL was recommended to be performed at the transplant center

at the earliest opportunity and favored over induced sputum. Since the patient resided approximately 100 km from our transplant center, and due to SARS-CoV-2 infection, number of visits to the hematology center was significantly reduced.

5 | Question 2: What Is Your Differential Diagnosis for Tree-in-Bud Lesions in This Setting?

MS: Over the following month, the tree-in-bud lesions increased (Figure 3). These lesions are not pathognomonic for a specific organism. Moreover, their evaluation must take into consideration the immune status of the patient. SARS-CoV-2 infection and alternative respiratory viral pathogens remain as possibilities. In this case, the subacute presentation raises suspicion for an atypical infection, with less likelihood of pyogenic bacterial pathogens which may progress more rapidly and acutely. In the context of immunosuppression, I think the differential diagnosis would be expanded to include consideration of fungal and mycobacterial infections. Given the recent positive test for SARS-CoV-2, my primary concerns would be the exclusion of pulmonary aspergillosis and mycobacterial infections (both tuberculous and nontuberculous). The radiological pattern is not particularly typical of pulmonary tuberculosis (TB), but in an immunosuppressed host, a "classic pattern" may not be seen: lesions often present as less cavitated with a greater tendency for dissemination throughout the body. Beyond the radiological findings, it is crucial to establish a diagnosis through BAL or lung biopsy at this stage.

6 | ID Problem No. 3: Tuberculosis in CAR T-Cell Therapy

After 4 months of relapsing infection, a SARS-CoV-2 test was performed and returned negative. The patient had no productive cough and 2 days later a bronchoscopy was performed: a *Mycobacterium tuberculosis* (MTB) nucleic acid amplification test (NAAT) was positive for detection of MTB, with no mutations seen in the *rpoB* gene, suggesting rifampin susceptibility. Ten days later, the mycobacterial culture turned positive for MTB as well. The complete drug susceptibility testing (DST) was performed using a molecular antibiogram, which did not detect any mutations in the following genes: *rpoB* (rifampicin), *katG* (isoniazid), *gyrA* (fluoroquinolones), and *rrs* (streptomycin). A blood culture for mycobacteria was performed, yielding a negative result and the PET scan revealed no other potential sites of infection, thus excluding the presence of disseminated disease.

7 | Panel Discussion: Tuberculosis in CAR T-Cell Therapy

MS: Pulmonary TB can present with a wide variety of radiologic presentations. While upper lobe cavitary lesions often lead clinicians to consider TB in the differential, alternative presentations can range from lobar infiltrates to tree-in-bud patterns or fibronodular lesions and can be diagnostically challenging. In many instances, epidemiological risk factors are the only clue to considering TB in the differential. This patient was born in Italy, without known travel to endemic areas, no history of homelessness or incarceration. Consequently, this patient lacks typical epidemiologic risk factors for TB infection, lowering the pretest probability and adding to the diagnostic challenge. Presumably, there were unrecognized exposures which is not uncommon and raises questions about the need for TB screening prior to intensive immunosuppressive chemotherapeutics or biologics—an area of ongoing research with limited formal guidelines. In contrast, in a person with underlying TB infection (i.e., latent TB infection), immunosuppression constitutes a host risk factor for disease progression. In our case instance, the patient had normal lymphocyte count, he reported no productive cough, and there was relatively modest pulmonary involvement, and this could reflect an early reactivation. Sputum induction or more invasive tests such as BAL are often required for specimen acquisition when pulmonary TB is on the differential and are sometimes able to detect lower bacterial burdens. This scenario is not uncommon, particularly in immunosuppressed hosts, where pulmonary involvement can be more limited despite fairly extensive TB disease elsewhere in the body.

New tests, like the urinary antigen test (urinary lipoarabinomannan—LAM), endorsed by WHO for persons living with HIV, showed promise especially for diagnosis of disseminated forms of disease or for increasing diagnostic yield when used in conjunction with respiratory testing [8]. Whether such approaches could be used to more rapidly diagnose MTB in other populations, including those with other forms of immunosuppression, is an area of ongoing study [9, 10], but such non-site-specific tests may, in the near, future increase the ability to detect other forms of TB, particularly when obtaining biospecimens from the site of disease is challenging. In addition, there is an emerging pipeline of low-complexity and moderate complexity NAAT tests with improved diagnostic sensitivity and turnaround times, and with some of them suitable for near-care and possibly point-of-care usage. Moreover, these molecular platforms also offer the ability to detect a range of drug-resistance mutations, offering rapid assessment of drug resistance to first- or second-line anti-mycobacterial agents [11]. Finally, oral swabs which detect TB biomass on the tongue, may offer another potential diagnostic alternative in cases when sputum-based testing or more invasive testing (BAL, sputum induction) are not feasible [12].

8 | Question 3: How Would You Treat TB in This Case?

MS: Given the knowledge of rifampin sensitivity, I would likely start with a conventional regimen for drug-sensitive TB which includes rifampin, isoniazid, pyrazinamide, and ethambutol (RHZE), while awaiting more comprehensive DST. The therapy goals in this case encompass both short- and long-term objectives. Initially, during the early treatment phase (i.e., intensive phase of a standard RHZE regimen), the primary aim is to rapidly reduce the bacterial burden of mycobacteria. Isoniazid and fluoroquinolones are drugs with strong early bactericidal activity (EBA) and contribute most to the initial reduction in bacterial burden. Conversely, rifampicin is the primary first-line drug with sterilizing properties, and is central to the regimen's ability to achieve long-term cure. However, there is increasing data that standard rifampicin recommended dosages may not be adequate

for all patients [13]. Moreover, optimizing treatment, potentially through higher doses of rifampicin or related drugs with better PK/PD parameters like rifapentine, can improve the rifamycin's EBA leading to shorter times to culture conversion, and potentially to treatment shortening [14]. While not a guideline recommended this approach, in rare instances where a patient with drug-sensitive TB is acutely ill or hemodynamically unstable, some TB clinicians will consider inclusion of a fluoroquinolone to the original regimen, and despite limited data, the REMox trial also highlighted a quicker clinical and microbiological response by substituting ethambutol with moxifloxacin, although it did not demonstrate non-inferiority of the 4-month regimen compared to the 6-month regimen [15].

Long-term objectives primarily focus on curing TB (i.e., sterilizing persistent organisms) and preventing relapses. Rifamycins (and newer agents such as bedaquiline) are key components to modern antituberculous regimens that have sterilizing activity and allow for relatively shorter courses of treatment and are the backbone of the later continuation phase of standard therapy. The overall treatment duration therefore depends on the regimen utilized, as well as the patient's clinical and microbiological response. While immunosuppression can influence treatment duration, it is not the sole determinant. Prolonged therapy may be considered, if necessary, especially in cases of high bacterial burden at diagnosis or slow microbiological response. It should be noted that new treatment guidelines for drug-sensitive and drug-resistant TB are now available and they highlight alternative and shorter options as well [16]. For example, studies have demonstrated that a regimen (HPMZ) comprising isoniazid (H), pyrazinamide (P), rifapentine (P) and moxifloxacin (M) for 4 months is non-inferior to the standard 6-month regimen (HRZE), with faster microbiological clearance [17]. Experience with these alternative approaches is growing, but there may be trade-offs when considering pill burden, interactions, and tolerability with potential for overall treatment shortening [18].

9 | Clinical Case: TB in CAR T-Cell Therapy

After the diagnosis, the patient initiated antitubercular therapy with the RHZE regimen, with drug dosages calculated based on a body weight of 64 kg: rifampin 600 mg, isoniazid 300 mg, pyrazinamide 1500 mg, and ethambutol 1200 mg.

At that time the patient was on low-dose steroid treatment for B-symptoms of lymphoma. Few weeks after starting rifampicin, a worsening of fever was observed, and after a diagnostic work up for all other co-pathogens resulted negative, fever was attributed to the rifampicin-steroid interaction with potential lowering of steroid blood levels. Thus, the steroid dose was increased and the patient became afebrile and reported improvement of general clinical conditions.

After 1 month of antitubercular treatment, a new sputum culture was performed, still growing MTB, but after 28 days of incubation. A subsequent sample was promptly collected for culture after the positive result was reported. This positive result after 1 month of treatment did not raise suspicion of drug resistance, as DST had not identified any resistance-associated mutations and patient's compliance was confirmed. The drug dosages had been calculated based on the patient's weight, and there were no concerns regarding poor drug absorption. Consequently, the treatment regimen was not modified at that time. In addition, 5 months have passed since the CAR-T indication was made and a re-evaluation PET scan started to show a slow lymphoma progression, making it increasingly important not to delay CAR T-cell therapy any further.

10 | Panel Discussion: How Long After TB Treatment Initiation Can We Consider CAR T-Cell Administration?

Joshua Hill: Once again, there is no predetermined waiting period. Investigations into CAR T-cell treated patients with pre-existing infections have not demonstrated a significant exacerbation of the infection post-CAR T-cell therapy; however, stringent monitoring and optimized treatment were imperative [19]. Moreover, it is crucial to recognize in this particular case that the risk of an unfavorable outcome associated with lymphoma was likely greater than the risk of pulmonary TB progression. Effective medications are available for TB, and both the disease volume and symptoms were not markedly severe. Consequently, in consideration of these factors, I would recommend proceeding with CAR T-cell therapy within a short timeframe, ranging from a few weeks to a month.

11 | Panel Discussion: Managing Rifampin Interactions During CAR-T

MS: As was noted, TB is typically treatable even in the setting of advanced immunosuppression with careful therapeutic considerations and close monitoring, and should not necessarily preclude management of other conditions. In this case, given the continued culture positivity, I would begin by ensuring the effectiveness of the current TB treatment regimen—this includes evaluation of DST for first- and second-line drugs to ensure there is no acquired drug resistance to one or more components of the TB regimen, and conducting therapeutic drug level monitoring. Slow or inadequate microbiological responses can be seen with low-drug levels, particularly for rifamycins and for isoniazid. The patient had drug-susceptibility to all first-line drugs, and assuming drug levels were adequate, the primary considerations and challenges have to do with rifamycin drug interactions. The primary interactions of rifampicin do not concern CAR T-cell infusion, but rather other drugs used in this context, particularly steroids and chemotherapy. As mentioned earlier, rifampicin is the only first-line drug that acts to sterilize the pathogen. While TB can be cured even without rifampicin, the treatment duration must be extended to 12–24 months with most historically used alternative regimens (that relied on drugs such as isoniazid, moxifloxacin, ethambutol, and pyrazinamide). It should be noted that new short-course regimens (i.e., 6-month durations) that utilize newer agents such as bedaquiline (an ATP sythenase inhibitor) and pretomonid (a nitroimidazole) have emerged and are now recommended for multi-drug resistant TB [16]. Whether these newer regimens (e.g., BPaL/M, i.e. bedaquiline, pretomanid, linezolid, and moxifloxacin) should also be utilized for rifampin-intolerant or rifampin-sparing regimens is unclear. Nonetheless, given the promising outcomes when used for rifampin-resistant

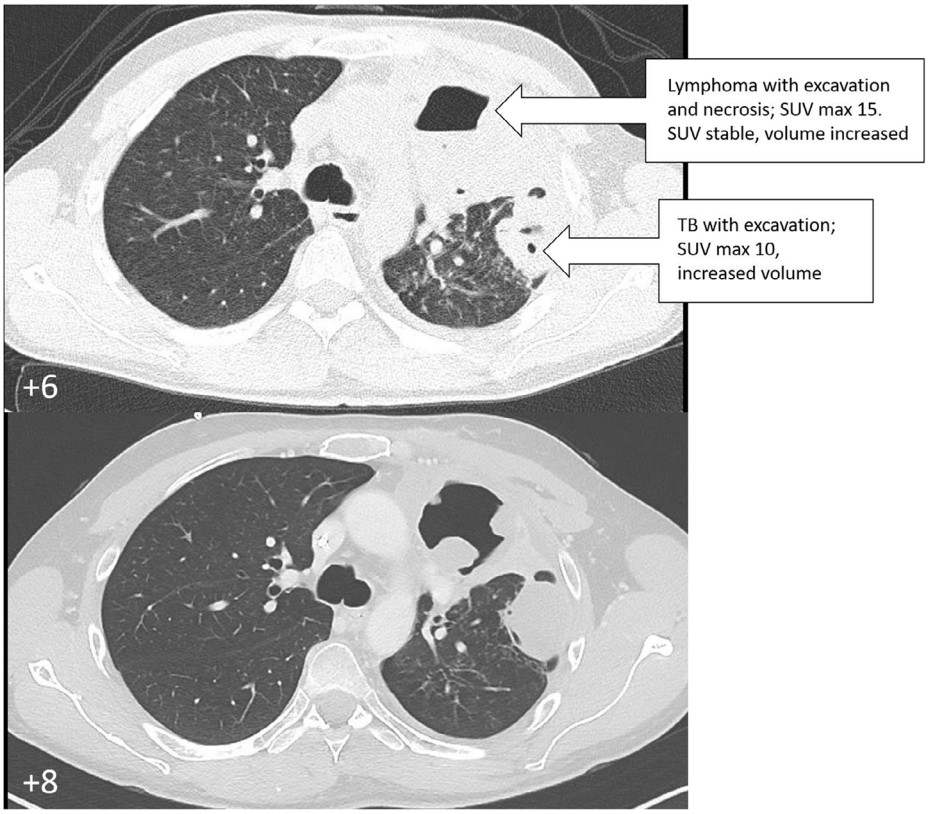

FIGURE 4 | Upper panel: CT scan, performed after nearly 11 weeks of antitubercular therapy: a radiological progression of lymphoma and TB lesions is shown, with the appearance of excavations. CAR T-cell therapy was administered 2 weeks later. Lower panel: 1 month after CAR-T the CT scan showed an evolution in the radiological findings: the lesion where the lymphoma used to be increased in volume, mainly excavated component, which could be an effect of CAR T-cell therapy. The TB lesion became more consolidated.

TB disease, usage of these regimens is increasing in many settings for rifampin-sparing regimens (i.e., due to pharmacologic interactions), and appears to be well tolerated with good clinical outcomes [20].

Indeed, the aforementioned BPaL/M regimen has been studied as a 6-month therapeutic regimen, has its own unique set of potential adverse effects, making it increasingly necessary to individualize the choice of therapeutic regimen for each patient [21]. For example, bedaquiline can cause QT prolongation, and implementation requires close interval EKG monitoring. While linezolid is dosed lower than for gram positive infections, prolonged usage commonly results in cytopenias or neuropathy that can be treatment limiting; this risk is mitigated by close linezolid drug level monitoring to prevent accumulation [22].

12 | Clinical Case: CAR-T

After completing 2 months of 4-drug standard antitubercular therapy (i.e., intensive phase), the patient was switched to the combination of rifampicin and isoniazid for the continuation phase. However, a PET scan performed 3 weeks after the switch, showed a radiological progression in lymphoma and in TB lesions, with the appearance of excavations (Figure 4, upper panel). Given the critical situation after almost 3 months of antitubercular therapy the main concern was the progression of the lymphoma, and it was decided to proceed to CAR T-cell therapy. At that point, the sputum culture was negative for MTB, although the final result would only become available 2 weeks after the procedure.

A conditioning chemotherapy was administered (cyclophosphamide 500 mg/m^2 on days 1–3 + fludarabine 30 mg/m^2 on Days 1–3), and a few days later, CAR T-cell infusion was performed (axicabtagene ciloleucel, volume 68 mL, cells 0.4–2 × 10^8).

Before the initiation of lymphodepleting therapy, the antitubercular regimen was modified by replacing rifampin with levofloxacin, in order to mitigate the possible pharmacological interactions between rifampin and cyclophosphamide, as well as corticosteroid therapy; levofloxacin was chosen over moxifloxacin for its anti-pseudomonas activity, a quite common pathogen in neutropenic fever in our epidemiological setting.

On Day +1 after CAR-T, the patient developed a Grade 1 cytokine release syndrome (CRS), treated with two doses of tocilizumab. On Day +3, the patient developed neurotoxicity with Grade 3 immune effector cell-associated neurotoxicity syndrome (ICANS), for which one dose of anakinra was administered, but on Day +5, clinical deterioration with encephalitis, aphasia, and respiratory distress occurred, leading to the patient's transfer to the intensive care unit.

Due to concurrent ICANS with epileptic seizures, all neurotoxic drugs, including levofloxacin and isoniazid, were discontinued,

and empirical therapy with meropenem and amikacin (both active also against MTB) was prescribed for febrile neutropenia. Starting from Day +7, there was an improvement in the patient's general condition, antibiotic therapy was discontinued, steroid therapy was gradually reduced and eventually discontinued, and antitubercular therapy with rifampicin and isoniazid was resumed.

Upon resolution of febrile neutropenia, prophylaxis with micafungin was discontinued, while other prophylactic regimens (TMP/SMX and valacyclovir) were continued [23]. The patient was discharged home and 2 weeks later (1 month after CAR T-cell therapy) a re-evaluation CT scan was performed (Figure 4, lower panel), showing an evolution in the radiological findings: the lesion where the lymphoma used to be increased in volume, mainly its excavated component, which could be an effect of CAR T-cell therapy. The TB lesion became more consolidated.

At that time, the patient was clinically stable, with negative sputum culture and had completed 4 months of standard antitubercular therapy, including 2 months of the continuation phase during which a rifampicin-sparing regimen was administered for 1 week, followed by a 1-week interruption of therapy before resuming standard treatment, making it necessary to consider a prolonged treatment course of antitubercular treatment.

13 | Question 4: How We Should Evaluate These Radiological Changes?

JH: The main lesion, attributed to TB, appears to be responding to therapy, but this does not rule out the possibility of a new lesion from TB that developed due to ongoing cytopenia and immune suppression (Figure 4). A concept often utilized in the realm of invasive aspergillosis is that of a "mixed response," in which one can see areas of improvement and worsening radiologically in the context of ongoing immunosuppression despite appropriate therapy.

Among the differential diagnoses, *Pseudomonas aeruginosa* pneumonia is less likely because the patient is not clinically compromised to that extent. A recurrence of lymphoma cannot be completely ruled out, although the patient appears to have responded well to CAR-T cell therapy. *Nocardia* could be an alternative diagnosis. In this situation, I would consider performing a BAL, or, if the patient is clinically stable, a follow-up CT scan (after 2 weeks) followed by BAL if there is continued progression.

MS: From the TB standpoint, I would add that the patient has undergone 4 months of therapy, and while disease progression is possible, it is uncommon during treatment if the organism is susceptible and there are adequate drug levels. It is quite common for patients to have drugs suspended, particularly due to toxicity issues, and it is uncommon to see disease progressions when the treatment interruptions are short, and particularly after achieving microbiological responses (i.e., culture negativity). I will also add that paradoxical inflammatory reactions are common with various forms of TB, and we do see worsening radiographic appearance in some instances despite adequate microbiological therapy; this phenomenon is not uncommon in scenarios where there has been immune reconstitution following cessation of immunosuppressive medications.

14 | ID Problem No. 4: Fungal infection After CAR T-Cell Therapy

Few days after the CT scan, and more than 1 month after CAR-T-cell therapy, the patient reported a new worsening of cough and sputum. A molecular testing for TB returned negative, while sputum culture yielded positive result for *Aspergillus niger*, which was confirmed in a second sample. The patient was then admitted to the infectious diseases ward with suspicion of invasive aspergillosis or aspergilloma, that is, aspergillus infecting a pre-formed cavity.

15 | Panel Discussion: Invasive Fungal Infections After CAR T-Cell Therapy

JH: This is a fungal infection with a relatively late onset, occurring over a month after CAR T-cell therapy. However, the patient is indeed at risk for fungal infection, having developed and been treated for CRS and ICANS with high doses of corticosteroids and immunosuppressants. In addition, he previously experienced a prolonged SARS-CoV-2 infection, increasing the risk for developing COVID-19-associated pulmonary aspergillosis (CAPA). The TB infection has likely also impacted the airways. Studies focusing on invasive fungal infections in CAR T-cell therapy recipients have demonstrated an incidence of around 5%, often with unfavorable outcomes [24]. It is challenging to determine whether this is an aspergilloma, but this requires treatment in this context.

DN: In your center, what is the antifungal prophylaxis regimen used? In our center, for this type of patients, we typically administer posaconazole for the first 4 weeks after CAR T-cell therapy.

MM: In our center, an echinocandin is used during neutropenia, continued until neutrophil recovery and steroid tapering, particularly if neutropenia persists. In this case, the patient received micafungin during hospitalization, but upon discharge, steroid therapy was discontinued and neutropenia had resolved, so no azole prophylaxis was considered.

JH: At our center, we use fluconazole during neutropenia, which is then discontinued. However, if a patient has received more than 3 days of corticosteroids for the treatment of CRS or ICANS, they are considered at higher risk for developing a fungal infection, and thus mould active prophylaxis is initiated for at least a month after the last dose of immunosuppressive therapy. Nonetheless, this remains a controversial issue, as it is a debatable approach due to the high number needed to treat. The policies vary between the centers. The issue of IFD after CAR-T has been reviewed recently, reporting the rate of invasive aspegillosis of 3% [25].

16 | Clinical Case: antifungal treatment

Invasive pulmonary aspergillosis was diagnosed based on the aforementioned findings in addition to a positive serum galac-

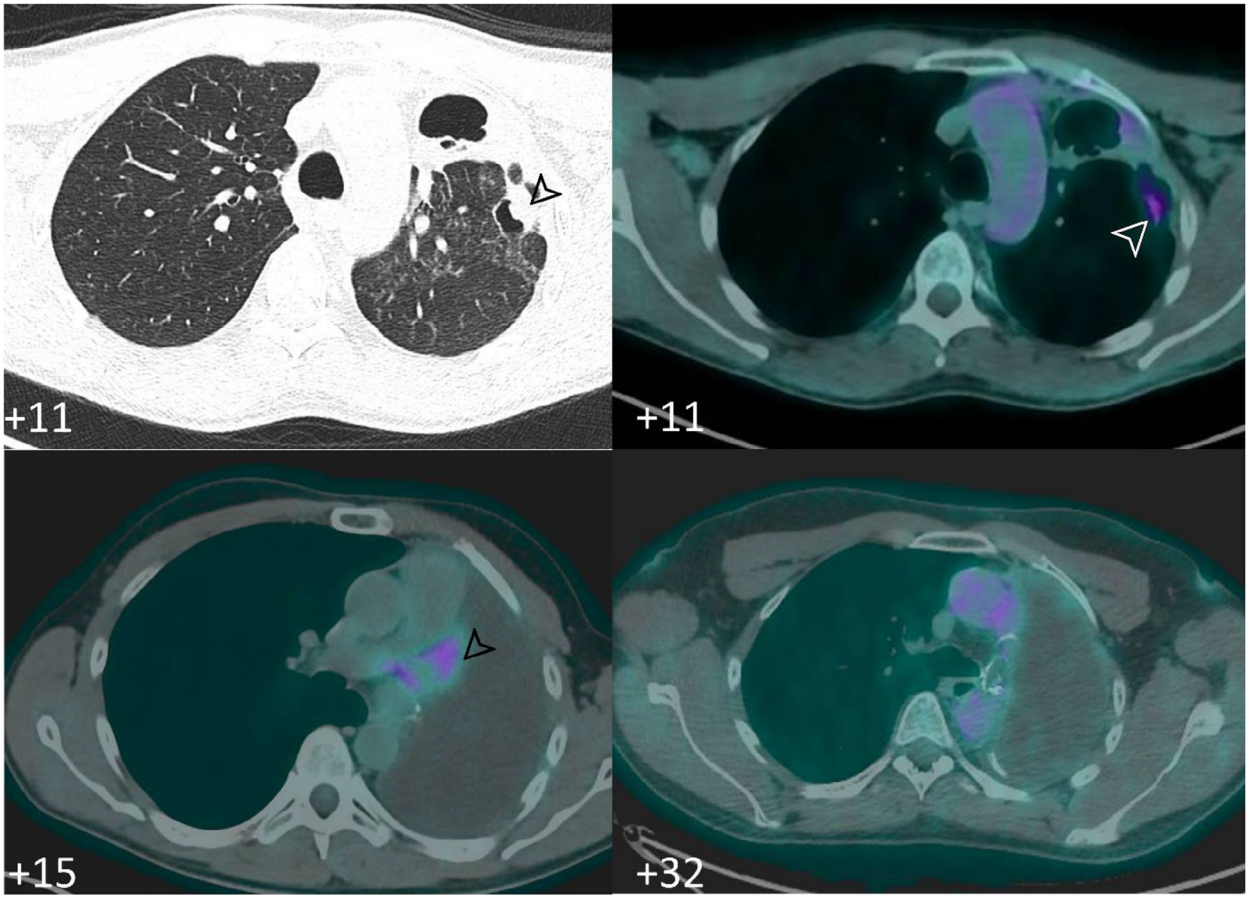

FIGURE 5 | Upper panel: PET scan performed 1 month after stopping antitubercular treatment (Month +11) showed a slight metabolic increase compared with previous controls at the level of the left anterior lung lesion (arrows). Pulmonary lobectomy performed 2 weeks later (at Month +11.5) due to clinical worsening with extensive hemoptysis. Lower panel: PET scan performed after pulmonary lobectomy showed a residual uptake, due to either recent surgery or residual fungal infection. A PET scan performed almost 2 years after CAR-T (Month +32) did not show any sign of infection or hematologic disease.

tomannan (optical density index, ODI 1.9). Treatment with amphotericin B was started. Rifampicin was replaced with moxifloxacin in order to limit interactions with azoles and after 5 days, isavuconazole was added, and after another 5 days of combination therapy and having documented achieving sufficient serum levels of isavucoanzole (1.3 mg/L), monotherapy with isavuconazole was continued. The patient was then discharged home with oral isavuconazole. Subsequent blood levels of isavucoanzole were between 1.5 and 3.5 mg/L.

17 | Question 5: How Long Should we Treat Invasive Aspergillosis in CAR T-Cell Patients?

JH: In this particular scenario, the precise duration of treatment cannot be predetermined due to the unpredictable nature of immune system recovery. There have been reported instances where immune reconstitution exhibits a biphasic pattern, characterized by an initial improvement followed by a subsequent onset of lymphopenia and neutropenia. Therefore, it is imperative to conduct radiographic monitoring of the patient using CT scans at intervals of 3–4 weeks to ascertain the appropriate treatment duration. Nonetheless, it is anticipated that weeks to months of therapy will likely be required.

18 | Clinical Case: Follow-Up

The antitubercular treatment was terminated relatively early, after a total duration of 6 months, due to patient's strong reluctance to continue therapy due to concerns regarding toxicity. At that point, the patient was counselled regarding increased risk of TB relapse given the need to use a rifamycin sparing regimen and the slow time to culture conversion (2 months), which are considered risks factor for recurrence.

One month after the discontinuation of antitubercular treatment, a PET/CT scan revealed still active pulmonary lesions (figure 5, upper panel), with a slight metabolic increase compared with previous control at the level of the left anterior lung lesion.

In the following week, the patient experienced an episode of hemoptysis, leading to a multidisciplinary discussion aimed at evaluation of the risk of successive massive bleeding considering the lesion's extreme proximity to the left pulmonary artery. A surgical intervention with an atypical resection was planned and was performed shortly after, but was converted to full pulmonary lobectomy during surgery. The histopathological examination of the excised lobe revealed a chronic inflammatory focus with necrotic-abscess formation, cavitation, and a fistulous tract

involving the bronchial wall, accompanied by a giant cell granulomatous inflammatory response. Histochemical staining with Grocott and PAS did not detect the presence of fungal hyphae. No significant alterations suggestive of neoplastic processes were observed in the area. Culture testing for bacteria, fungi, and mycobacteria were performed on the intraoperative specimen and all yielded negative results.

Isavuconazole was discontinued after 8 months of therapy, 2 months later than originally planned due to residual uptake observed on PET imaging (Figure 5, lower panel) [26], although the metabolic activity could have been due to either recent surgery or residual fungal infection. A repeated PET scan 2 months after the discontinuation was negative. Lymphocyte cell counts performed 16 months after CAR-T (CD3+ 280, CD19+ 0, CD4+ 168, and CD8+ 109) demonstrated that waiting for complete immune reconstitution before discontinuing antifungal treatment would have resulted in prolonged treatment course. Indeed, the patient achieved a CD4+ T-cell count above 200 only 1 year later, that is, 24 months after CAR-T, while hypogammaglobulinemia had not yet resoled at the time (IgM levels below the limit of detection, IgG 3.51 g/L and IgA 0.21 g/L). In these 2 years following CAR-T, the patient has remained in good clinical conditions, with lymphoma in complete remission. The last PET scan did not show any sign of infection or relapse (Figure 5, lower panel).

Author Contributions

Monica Melchio: conceptualization, writing. **Joshua A. Hill**: conceptualization, writing. **Maunank Shah**: conceptualization, writing. **Dionysios Neofytos**: conceptualization. **Massimiliano Gambella**: reviewing and editing. **Anna Maria Raiola**: reviewing and editing. **Emanuele Delfino**: reviewing and editing. **Elisa Balletto**: reviewing and editing. **Emanuele Angelucci**: reviewing and editing. **Matteo Bassetti**: reviewing and editing. **Malgorzata Mikulska**: conceptualization, writing, reviewing and editing. All authors were involved for critical revision of the manuscript and approved the final version before submission.

Acknowledgments

Open access publishing facilitated by Universita degli Studi di Genova, as part of the Wiley - CRUI-CARE agreement.

Ethics Statement

A written informed consent was obtained from the patient.

Conflicts of Interest

Joshua A. Hill reports relevant consultancy for Allovir, Century Therapeutics, Exevir, Geovax, Karius, Moderna, Medscape, Sanofi, and SentiBio; and research support from Geovax, Gilead, and Takeda. Matteo Bassetti has received funding for scientifc advisory boards, travel, and speaker honoraria from Cidara, Gilead, Menarini, MSD, Mundipharma, Pfizer, and Shionogi. The other authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. P. J. Hayden, C. Roddie, P. Bader, et al., "Management of Adults and Children Receiving CAR T-Cell Therapy: 2021 Best Practice Recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE) and the European Haematology Association (EHA)," *Annals of Oncology* 33, no. 3 (2022): 259–275, https://doi.org/10.1016/j.annonc.2021.12.003.

2. V. Dioverti, Z. El Boghdadly, Z. Shahid, et al., "Revised Guidelines for Coronavirus Disease 19 Management in Hematopoietic Cell Transplantation and Cellular Therapy Recipients (August 2022)," *Transplantation and Cellular Therapy* 28, no. 12 (2022): 810–821, https://doi.org/10.1016/j.jtct.2022.09.002.

3. S. Cesaro, M. Mikulska, H. H. Hirsch, et al., "Update of Recommendations for the Management of COVID-19 in Patients With Haematological Malignancies, Haematopoietic Cell Transplantation and CAR T Therapy From the 2022 European Conference on Infections in Leukaemia (ECIL 9)," *Leukemia* 37, no. 9 (2023): 1933–1938, https://doi.org/10.1038/s41375-023-01938-5.

4. ECIL-10. Community-acquired respiratory virus (CARV) infections in HM and HCT patients 2024: Update of 2019 recommendations, https://www.ecil-leukaemia.com/images/ecil-10/CARVs_Update_2024_ECIL-10_final_4.pdf.

5. P. Ljungman, G. Tridello, J. L. Piñana, et al., "Improved Outcomes Over Time and Higher Mortality in CMV Seropositive Allogeneic Stem Cell Transplantation Patients With COVID-19; an Infectious Disease Working Party Study From the European Society for Blood and Marrow Transplantation Registry," *Frontiers in Immunology* 14 (2023): 1125824, https://doi.org/10.3389/fimmu.2023.1125824.

6. Y.-J. Kim, A. Waghmare, H. Xie, et al., "Respiratory Viruses in Hematopoietic Cell Transplant Candidates: Impact of Preexisting Lower Tract Disease on Outcomes," *Blood Advances* 6, no. 18 (2022): 5307–5316, https://doi.org/10.1182/bloodadvances.2021004915.

7. M. Mikulska, D. Testi, C. Russo, et al., "Outcome of Early Treatment of SARS-CoV -2 Infection in Patients With Haematological Disorders," *British Journal of Haematology* 201, no. 4 (2023): 628–639, https://doi.org/10.1111/bjh.18690.

8. World Health Organization, "WHO Consolidated Guidelines on Tuberculosis: Module 3: Diagnosis," accessed June 12, 2025, https://www.who.int/publications/i/item/9789240107984.

9. M. Shah, W. Ssengooba, D. Armstrong, et al., "Comparative Performance of Urinary Lipoarabinomannan Assays and Xpert MTB/RIF in HIV-Infected Individuals," *Aids* 28, no. 9 (2014): 1307–1314, https://doi.org/10.1097/QAD.0000000000000264.

10. World Health Organization, "Lateral Flow Urine Lipoarabinomannan Assay (LF-LAM) for the Diagnosis of Active Tuberculosis in People Living With HIV: Policy Update 2019," accessed November 6, 2024, https://iris.who.int/handle/10665/329479.

11. E. Maclean, M. Kohli, S. F. Weber, et al., "Advances in Molecular Diagnosis of Tuberculosis," *Journal of Clinical Microbiology* 58, no. 10 (2020): e01582–e01619, https://doi.org/10.1128/JCM.01582-19.

12. A. Andama, G. R. Whitman, R. Crowder, et al., "Accuracy of Tongue Swab Testing Using Xpert MTB-RIF Ultra for Tuberculosis Diagnosis," *Journal of Clinical Microbiology* 60, no. 7 (2022): e0042122, https://doi.org/10.1128/jcm.00421-22.

13. N. F. Maranchick and C. A. Peloquin, "High Time for Higher-Dose Rifampin," *Clinical Infectious Diseases* 81 (2025): 143–144, https://doi.org/10.1093/cid/ciaf005.

14. S. E. Dorman, P. Nahid, E. V. Kurbatova, et al., "Four-Month Rifapentine Regimens With or Without Moxifloxacin for Tuberculosis," *New England Journal of Medicine* 384, no. 18 (2021): 1705–1718, https://doi.org/10.1056/NEJMoa2033400.

15. S. H. Gillespie, A. M. Crook, T. D. Mchugh, et al., "Four-Month Moxifloxacin-Based Regimens for Drug-Sensitive Tuberculosis," *New England Journal of Medicine* 371, no. 17 (2014): 1577–1587, https://doi.org/10.1056/NEJMoa1407426.

16. J. J. Saukkonen, R. Duarte, S. S. Munsiff, et al., "Updates on the Treatment of Drug-Susceptible and Drug-Resistant Tuberculosis: An Official ATS/CDC/ERS/IDSA Clinical Practice Guideline," *American Journal of Respiratory and Critical Care Medicine* 211, no. 1 (2025): 15–33, https://doi.org/10.1164/rccm.202410-2096ST.

17. S. E. Dorman, P. Nahid, E. V. Kurbatova, et al., "Four-Month Rifapentine Regimens With or Without Moxifloxacin for Tuberculosis," *New England Journal of Medicine* 384, no. 18 (2021): 1705–1718, https://doi.org/10.1056/NEJMoa2033400.

18. J. K. Louie, R. Agraz-Lara, G. E. Velásquez, A. Phillips, and J. D. Szumowski, "Experience With Four-Month Rifapentine and Moxifloxacin–Based Tuberculosis Treatment in San Francisco," *Open Forum Infectious Diseases* 11, no. 4 (2024): ofae178, https://doi.org/10.1093/ofid/ofae178.

19. J. A. Hill and S. K. Seo, "How I Prevent Infections in Patients Receiving CD19-Targeted Chimeric Antigen Receptor T Cells for B-Cell Malignancies," *Blood* 136, no. 8 (2020): 925–935, https://doi.org/10.1182/blood.2019004000.

20. C. A. Haley, M. C. Schechter, D. Ashkin, et al., "Implementation of Bedaquiline, Pretomanid, and Linezolid in the United States: Experience Using a Novel All-Oral Treatment Regimen for Treatment of Rifampin-Resistant or Rifampin-Intolerant Tuberculosis Disease," *Clinical Infectious Diseases* 77, no. 7 (2023): 1053–1062, https://doi.org/10.1093/cid/ciad312.

21. F. Conradie, A. H. Diacon, N. Ngubane, et al., "Treatment of Highly Drug-Resistant Pulmonary Tuberculosis," *New England Journal of Medicine* 382, no. 10 (2020): 893–902, https://doi.org/10.1056/NEJMoa1901814.

22. N. F. Maranchick, C. A. Peloquin, and C. A. Haley, "Linezolid Dosing and Pharmacokinetics in North American Patients With Tuberculosis," *Clinical Infectious Diseases* (2025): ciaf027, https://doi.org/10.1093/cid/ciaf027. Epub ahead of print.

23. Z. Shahid, T. Jain, V. Dioverti, et al., "Best Practice Considerations by The American Society of Transplant and Cellular Therapy: Infection Prevention and Management After Chimeric Antigen Receptor T Cell Therapy for Hematological Malignancies," *Transplantation and Cellular Therapy* 30, no. 10 (2024): 955–969, https://doi.org/10.1016/j.jtct.2024.07.018.

24. G. Haidar, K. Dorritie, R. Farah, T. Bogdanovich, M. H. Nguyen, and P. Samanta, "Invasive Mold Infections After Chimeric Antigen Receptor–Modified T-Cell Therapy: A Case Series, Review of the Literature, and Implications for Prophylaxis," *Clinical Infectious Diseases* 71, no. 3 (2020): 672–676, https://doi.org/10.1093/cid/ciz1127.

25. J. S. Little, E. Kampouri, D. Z. Friedman, et al., "The Burden of Invasive Fungal Disease Following Chimeric Antigen Receptor T-Cell Therapy and Strategies for Prevention," *Open Forum Infectious Diseases* 11, no. 6 (2024): ofae133, https://doi.org/10.1093/ofid/ofae133.

26. A. Fernández-Cruz, R. E. Lewis, and D. P. Kontoyiannis, "How Long Do We Need to Treat an Invasive Mold Disease in Hematology Patients? Factors Influencing Duration of Therapy and Future Questions," *Clinical Infectious Diseases* 71, no. 3 (2020): 685–692, https://doi.org/10.1093/cid/ciz1195.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: tid70093-sup-0001-videoS1.mp4. **Supporting File 2:** tid70093-sup-0002-VisualAbstract.jpg.