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# Exogenous Interferon-γ Immunotherapy for Invasive Fungal Infections in Kidney Transplant Patients

**D. Armstrong-James[a,†], I. A. Teo[a,†], S. Shrivastava[b,d], M. A. Petrou[c], D. Taube[d], A. Dorling[b,d,e] and S. Shaunak[a,*]**

[a] *Department of Infectious Diseases and Immunity,* [b] *Department of Immunology,* [c] *Department of Medical Mycology and* [d] *West London Renal and Transplant Centre, Faculty of Medicine, Imperial College London & Imperial Healthcare NHS Trust London, London, UK,* [e] *Current address: MRC Centre for Transplantation, King's College London, Guy's Hospital, London SE1 9RT*
**Corresponding author: Professor Sunil Shaunak, s.shaunak@imperial.ac.uk*
† *These authors contributed equally to the work.*

**The incidence of invasive fungal infections (IFIs) in nonneutropenic solid organ transplant patients is increasing. We report our clinical experience with the use of interferon-γ (IFN-γ) immunotherapy in seven renal transplant patients who developed life threatening, disseminated IFIs refractory to conventional antifungal drug therapy. The infections were all microbiologically and histologically proven. The rapid cure of these disseminated infections with exogenous IFN-γ injections was not associated with impaired kidney allograft function despite the use of liposomal amphotericin B in all cases. No clinical toxicity from the IFN-γ immunotherapy was seen and no IFI relapsed during long-term follow-up. Our experience is both uncontrolled and in patients with unpredictable fungal infection-related outcomes. However, compared to standard approaches, the accelerated cure of life threatening, disseminated IFIs with 6 weeks of combination antifungal drug therapy and IFN-γ immunotherapy saved lives, retained allograft function and led to substantial cost savings in this small patient group.**

**Key words: Interferon-γ (IFN-γ) immunotherapy, invasive fungal infections, kidney transplantation**

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## Introduction

The incidence of invasive fungal infections (IFIs) in nonneutropenic solid organ transplant patients has been increasing in recent years. This reflects the larger populations of chronically immunosuppressed solid organ and stem cell transplant patients, and the use of new immunosuppressive drug regimens. US data shows a year-on-year increase in the incidence of invasive aspergillosis in solid organ transplant patients; 8% for lung; 5% for heart; 2% for liver; and 1% for kidney (1–9). Three classes of drugs have become core components of long-term immunosuppressive therapy for transplant patients; antiproliferative agents (mycophenolate mofetil [MMF] and azathioprine); calcineurin inhibitors [CNIs] (tacrolimus and cyclosporine A) and corticosteroids (10–13). In the case of stem cell transplant patients, 70% of IFIs develop postneutropenia and during the course of their long-term treatment (14). These associations suggest that T cell-mediated immune defects are becoming an increasingly important risk factor for IFIs. No diagnostically useful assay is currently available for identifying the subgroup of patients on modern immunosuppressive drug regimens who are at significantly increased risk of IFIs. The role of IFN-γ immunotherapy in this group of patients also remains to be defined (15–20).

Animal-based studies have shown that IFIs are associated with an impaired Th1 host immune response (21–23). Modern long-term transplant immunosuppressive drug regimens could therefore be predisposing patients to IFIs because they are designed to target adaptive Th-1-mediated allograft rejection. The result could be a cumulative failure to produce an effective IFN-γ-driven response to fungal pathogens. Our microarray-based studies in murine models of pulmonary aspergillosis show that clearance of the infection in immunocompetent mice requires an effective host Th1-orientated transcriptional program. Death in immunosuppressed mice was associated with a disordered transcriptional program characterized by increased expression of genes encoding for: (1) TNF-related proteins; (2) TNF signaling; and (3) proteins responsible for activation and expansion of Th2/Th17 CD4+ T cells. These mice had markedly reduced IFN-γ production in the infected lung (24).

In 2005, we published the case report of a renal transplant patient in whom disseminated cryptococcal disease was cured only after the addition of recombinant IFN-γ therapy (25). Five years later, he remains well. This experience led us to a preliminary investigation of the quantitative PBMC mRNA cytokine profiles of several patients with IFIs. Our preliminary results suggested an impaired IFN-γ response, an excessive IL-10 antiinflammatory response

and an inadequate TNF-α proinflammatory response (26). These cytokine differences were significant when IFI patients were compared with either hemodialysis patients (2-fold deficiency in IFN-γ and 330-fold excess of IL-10) or healthy normal individuals (5-fold deficiency in IFN-γ, 3.5-fold excess of IL-10 and 2-fold deficiency of TNF-α) as the control groups (paper in preparation). These and other observations (27,28) led us to evaluate the potential therapeutic benefit of IFN-γ immunotherapy in seven patients with life threatening, disseminated IFIs that were refractory to conventional antifungal drug therapy. These patients were seen in our renal transplant unit after our first case report experience as reported in late 2005 (25). We want to emphasize that this report relates only to patients with extreme forms of IFIs.

## Materials and Methods

### *Patients*

Patients were recruited from the West London Renal & Transplant Centre only. The patients described were seen in our renal transplant unit after our first case report experience as reported in late 2005 (25). Our unit performs >150 transplants/year and monitors >850 transplant patients. The study was approved by the UK's national research ethics committee. Patients gave informed consent.

### *Immunosuppressive therapy*

All but one of the patients who had a functioning kidney allograft was maintained on their baseline immunosuppressive drugs with appropriate drug dose adjustments when antifungal drugs were added. In patients with poor renal function (GFR<20 mL/min), immunosuppression was withdrawn.

### *IFN-γ injections*

Patients had started or restarted their antifungal drugs at least 10 days before recombinant human IFN-γ (Immunkin [Boehringer Ingelheim]; 200 μg subcutaneously; ×3/week) immunotherapy was added. It was given for 6 weeks.

### *Pathology*

Routine mycological culture techniques were used for all specimens. Antifungal drug susceptibility testing was performed according to CLSI standards (29,30).

## Results

The transplant and infection-related aspects of each case are summarized in Tables 1 and 2 respectively. Several additional and notable features of clinical importance for each case are now described.

### *Case 1*

This patient's kidney transplant was performed overseas. He was thought to be 'cured' of his aspergillus infection on two occasions that were 8 months apart with the use of prolonged courses (12 months in total) of voriconazole and caspofungin at a total cost of $100 000. After the first relapse, his cyclosporine A was stopped to aid fungal clearance. However, 5 months after stopping his antifungal drugs, he relapsed for a third time with the same infection. On each occasion, the diagnosis was histologically and microbiologically proven, and all radiologically identified collections were surgically drained. At no time, was there a change in the antifungal resistance profile of the isolate. After the introduction of IFN-γ immunotherapy, his CRP returned to normal in 3 weeks. Six weeks after stopping his antifungal drugs and IFN-γ immunotherapy, an 8-cm abscess at the lower pole of the transplanted kidney was surgically drained and excised. It was sterile on prolonged culture. He then remained well for 6 months before dying of a myocardial infarction. At autopsy, there was no evidence of any residual aspergillus infection. His creatinine (108–131 μmol/L) and eGFR (55–69 mL/min) remained stable during this 2-year period (Table 1).

### *Case 2*

This patient's kidney biopsy showed acute cellular rejection with focal intimal arteritis at 1 year. Over the next 6 years, his creatinine rose to 350 μmol/L (eGFR 17 mL/min). At 8 years posttransplant, he presented with a chronic nonproductive cough and ulcerating skin nodules. The dematiaceous fungus *Ochroconis gallopavum* was isolated from several body sites including lung. Tissue biopsies showed chronic granulomatous inflammation with multinucleated giant cells and focal necrosis. Antifungal drugs were started but his white cell count rose to $28 \times 10^9$/L and his CRP to 86 mg/L. His disease progression was manifest on CT scanning that showed radiological evidence of lung and cerebral involvement. He also developed a new and rapidly enlarging chest wall nodule. A diagnosis of disseminated phaehyphomycosis was made. As the reported mortality of cerebral *O. gallopavum* infection is 100%, his immunosuppression was withdrawn and hemodialysis started when his creatinine reached >400 μmol/L. IFN-γ immunotherapy was then started. He improved rapidly over the next 3 weeks with his CRP falling to 11 mg/L. A tissue biopsy and culture of a residual left flank nodule was performed 9 months after stopping IFN-γ immunotherapy. It showed an organizing granuloma with no residual infection. Thirty months after stopping his antifungal drugs, he remains well.

### *Case 3*

He underwent simultaneous pancreatic and renal transplantation. The pancreas failed 2 years later because of steroid resistant rejection. Four years later, his creatinine rose and a renal biopsy showed acute tubulitis. Despite treatment with pulsed intravenous methylprednisolone, he developed slowly progressive allograft dysfunction and became hemodialysis dependent 8 years later. His MMF and tacrolimus were stopped. Several weeks later, he developed malaise with fever and cough, and a CRP of 225 mg/L. A chest CT scan showed widespread nodular consolidation and cavitation from which *Aspergillus fumigatus* was repeatedly isolated. Antifungal drugs were started. His clinical condition continued to deteriorate with MRI brain scans showing several frontal and parietal lobe contrast

**Table 1:** Transplantation-related summary

| Patient | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Age | 42 M | 66 M | 36 M | 62 F | 43 M | 40 M | 48 F |
| Renal pathology | Accelerated nephrosclerosis | IgA nephropathy | Juvenile onset diabetes mellitus | Systemic lupus erythematosus | Nephrosclerosis | Nephrosclerosis | Cyclosporin nephropathy |
| Other clinical factors | No | No | Pancreatic & renal transplant | Asthma | no | BK nephropathy | Lung transplant 26 years previously |
| Transplant | LD | DD | DD pancreas & kidney | LD | LD | DD | DD – lung<br>LD – kidney |
| HLA mismatch | Unknown | (2,1,0) | (1,1,1) | (1,2,2) | (1,1,1) | (2,1,1) | (2,2,2) lung<br>(0,0,0) kidney |
| Immunosuppression—induction drugs | Unknown | Methylprednisolone | Methylprednisolone dacluzimab | Methylprednisolone dacluzimab | Methylprednisolone alemtuzumab | Methylprednisolone alemtuzumab | Methylprednisolone alemtuzumab |
| Immunosuppression—maintenance drugs | Cyclosporin A MMF prednisolone | Cyclosporin A azathioprine prednisolone *Postacute rejection* tacrolimus MMF prednisolone | Tacrolimus MMF | Tacrolimus MMF prednisolone | Tacrolimus *Post acute rejection* tacrolimus, MMF prednisolone | Tacrolimus *Post acute rejection* tacrolimus MMF prednisolone | Tacrolimus prednisolone |
| N° of rejection episodes (type) | 0 | 1 Tubulitis with focal arteritis at 12 m posttransplant. | 2 Pancreatic cellular at 24 m post transplant. Kidney tubulitis at 48 m posttransplant. | 1 AMR with TG at 8 m posttransplant. | 2 AMR with arteritis, followed by Banff 2a at 3 m posttransplant. | 1 Banff 1a at 6 m post transplant. | 1 AMR at 1 m post transplant. |
| Treatment of rejection | n/a | Methylprednisolone | Methylprednisolone | Rituximab | Methylprednisolone plasma exchange immunoglobulin (2 g/kg) rituximab | Methylprednisolone | Methylprednisolone plasma exchange immunoglobulin (2 g/kg) |
| Peri-infection immunosuppression | MMF prednisolone | None | None | Tacrolimus MMF prednisolone | Tacrolimus MMF prednisolone | Tacrolimus prednisolone<br>MMF – stopped because of BK nephropathy | Tacrolimus prednisolone |
| Renal outcome | Stable function<br>DWFG | GFR <20 mL/min prior to infection.<br>Graft lost secondary to withdrawal of IS. | Graft lost prior to infection. | Stable function | Graft lost due to rejection. | Stable function | DWFG |
| CMV IgG serology | Positive | Positive | Positive | Positive | Positive | Positive | Positive |
| CMV DNA PCR | Negative | negative | negative | negative | negative | negative | negative |
| Serum Cr/eGFR (μmol/L/mL/min) | | | | | | | |
| -Pre-IFN-γ therapy | 127/57 | 380/14 (on dialysis) | 471/12 (on dialysis) | 141/36 | 319/18 | 201/33 | 403/10 |
| -Post-IFN-γ therapy | 108/69 | not applicable | not applicable | 117/46 | 320/18 | 196/34 | 123/43 |
| -Long-term follow up | 131/55 | not applicable | not applicable | 155/35 | on dialysis | 195/33 | not applicable |

LD = living donor; DD = deceased donor; AMR = antibody-mediated rejection; DWFG = death with a functioning graft; IS = immunosuppression; TG = transplant glomerulopathy; n/a = not available.

**Table 2:** Infection-related summary

| Patient | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| EORCT Case Definition | Invasive aspergillosis | Phaeohypho-mycosis | Invasive aspergillosis | Invasive aspergillosis | Phaeohyphomycosis | Phaeohyphomycosis | Invasive candidiasis |
| Anatomical site confirmed | Peritransplant abscess, prostate | Lungs, skin, brain | Lungs, brain | Both lungs | Skin (multiple sites) | Skin (multiple sites) | Kidney, lungs blood |
| Organism | Aspergillus fumigatus | Ochroconis gallopavum | Aspergillus fumigatus | Aspergillus fumigatus | Altenaria malorum (A) & Pyrenothaeta romeroi (B) | Davidiella tassiana | Candida albicans |
| Antifungal drug MIC (μg/mL) ∗ = MFC | Amb = 0.12<br>CFG = 0.06*<br>ITZ = 0.06<br>PCZ = 0.007<br>VCZ = 0.5<br>5FC = >32<br>FCZ = >64 | Amb = 0.12<br>CFG = 0.06*<br>ITZ = 0.06<br>PCZ = 0.007<br>VCZ = 0.12<br>5FC = 1<br>FCZ = 64 | Amb = 0.12<br>CFG = 0.007*<br>ITZ = 0.06<br>PCZ = 0.03<br>VCZ = 0.12<br>5FC = 1<br>FCZ = 64 | Amb = 0.12<br>CFG = 0.007*<br>ITZ = 0.12<br>PCZ = 0.03<br>VCZ = 0.12<br>5FC = >32<br>FCZ = 64 | (A) (B)<br>Amb = 0.12; 0.5<br>CFG = 0.007*; 8*<br>ITZ = 0.01; 0.5<br>PCZ = 0.007; 0.25<br>VCZ = 0.007; 0.25<br>5FC = 0.5; 16<br>FCZ = 64; 64 | Amb = 0.25<br>CFG = 4*<br>ITZ = 0.12<br>PCZ = 0.06<br>VCZ = 0.12<br>5FC = >32<br>FCZ = 16 | Amb = 0.25<br>CFG = 0.007<br>ITZ = 0.03<br>VCZ = 0.007<br>PCZ = 0.007<br>5FC = 0.06<br>FCZ = 0.5 |
| Transplant to diagnosis time | 0.75 months | 96 months | 96 months | 9 months | 12 months | 20 months | 1 month |
| Symptoms to antifungal drugs | 1.5 weeks | 7 weeks | 4 weeks | 5 weeks | 6 weeks | 5 weeks | 1 week |
| First line antifungal drug therapy | Itraconazole 200 mg od po | LAmB (3 mg/kg) | Voriconazole 200 mg od po & caspofungin 50 mg od iv | Voriconazole 200 mg bd po | LAmB (3 mg/kg) | LAmB (5 mg/kg) | LAmB (4 mg/kg) |
| Second line antifungal drug therapy | Voriconazole 250 mg bd po & caspofungin 50 mg od iv & LAmB (3 mg/kg) | Voriconazole 200 mg bd iv | LAmB (3 mg/kg) | LAmB (3 mg/kg) | Itraconacole 200 mg bd po | Itraconazole 200 mg bd po and then posaconazole 400 mg bd po | Caspofungin 50 mg od iv |
| Time from antifungals to IFN-γ | 100 weeks | 9 weeks | 6 weeks | 6 weeks | 6 weeks | 6 weeks | 3 weeks |
| Relapse-free follow-up period | 6 months—died of a myocardial infarct | 36 months | 36 months | 24 months | 20 months | 12 months | 0.7 months |

EORTC = European organization for research and treatment of cancer; LAmB = liposomal amphotericin B (AmBisome); MIC = minimum inhibitory concentration; 5FC = 5-flucytosine; CFZ = caspofungin; FCZ = fluconazole; ITZ = itraconazole; PCZ = posaconazole; VCZ = voriconazole ∗ = MFC; minimum effective concentration.
This is the concentration at which casopfungin starts having an effect on the filamentous fungi. It never inhibits the mould so an MIC cannot be stated.
od = once daily; bd = twice daily; iv = intravenous; po = orally.

enhancing lesions. Disseminated aspergillosis was diagnosed and IFN-γ immunotherapy started. A week later, his CRP had fallen to 17 mg/L. A rapid clinical recovery followed with all his lung, cerebral and parietal lobe lesions resolving in 4 weeks. Thirty months after stopping his antifungal drugs, he remains well.

***Case 4***

Eight months after transplant, a kidney biopsy showed acute on chronic allograft glomerulopathy and was treated with one dose of rituximab (1 g). A month later, there was a repeated and heavy growth of *Aspergillus fumigates* from her sputum and bronchial biopsy. A CT chest scan showed multiple areas of dense opacification with ground glass opacification together with tree and bud changes. Despite treatment with antifungal drugs, her clinical condition deteriorated and her CRP increased to 228 mg/L. Nine days after IFN-γ immunotherapy was started, her CRP fell to 6 mg/L. After 6 weeks treatment with antifungal drugs and IFN-γ immunotherapy, a high-resolution CT chest scan showed complete resolution of all her lung infiltrates. Eighteen months later, she remains well with a creatinine of 155 μmol/L and eGFR of 35 mL/min (Table 1). She remains on her baseline immunosuppressive drugs.

***Case 5***

Three months after his transplant, his creatinine rose to 407 μmol/L (eGFR 23 mL/min). Successive renal biopsies showed severe antibody mediated rejection followed by acute cellular rejection. He was treated with pulse methylprednisolone, plasma exchange, high dose intravenous immunoglobulin and two doses of rituximab (1 g) (Table 1). His creatinine stabilized at 319 μmol/L (eGFR 18 mL/min). Nine months later, he presented with enlarging, tender, purple skin nodules on his arms and legs. Tissue biopsies showed fungal hyphae in numerous dermal abscesses with no multinucleate giant cells. A mixed growth of filamentous fungi were isolated that were identified as *Alterneria malorum* and *Pyrenochaeta romeroi.* Antifungal drugs were started but there was no change in the size or shape of the skin nodules. IFN-γ immunotherapy was therefore added and within a few days, his skin lesions started shrinking. He received 4.5 g of liposomal amphotericin B followed by 2 weeks of itraconazole (200 mg bd). Six weeks after starting IFN-γ, all his skin lesions had healed. A biopsy of his left forearm skin lesion at that time showed a marked dermal granulomatous reaction with dominant epitheloid macrophages and multinucleated giant cells. There was no growth on culture.

His renal function remained stable and he remained on his baseline immunosuppression. However, his creatinine then rose progressively over a period of several months. Multiple biopsies confirmed ongoing chronic active transplant glomerulopathy. No additional immunotherapy was given. His immunosuppression was withdrawn. He is now on hemodialysis. Twenty months later, he remains well.

***Case 6***

Six months after transplantation, his creatinine rose and a kidney biopsy showed acute cellular rejection that was treated with pulse methylprednisolone (Table 1). His creatinine stabilized at 140 μmol/L (eGFR 55 mL/min). Six months later, his creatinine rose to 201 μmol/L (eGFR 33 mL/min). A renal biopsy showed BK virus nephropathy. His MMF was stopped because of his BK virus nephropathy and he received intravenous immunoglobulin (2 g/kg). His creatinine stabilized.

Twenty months posttransplantation, he developed multiple skin nodules on his arms and legs. Histology of these nodules showed focal necrotizing abscesses in the dermis. The dermataceous fungus *Davidiella tassiana* was isolated. Despite treatment with antifungal drugs, his skin lesions increased in size and number. IFN-γ immunotherapy was added. His skin lesions started resolving rapidly and had all healed by 6 weeks. A repeat biopsy of a healed lesion showed a lymphoplasmacytic infiltrate with necrotizing epithelioid granulomas. There was no growth on culture. MRI scans confirmed complete resolution of the large subcutaneous fungal abscesses. Six months later, he remains well with a creatinine of 195 μmol/L and an eGFR of 33 mL/min (Table 1).

***Case 7***

She underwent a live-related kidney transplant 26 years after a double lung transplant and achieved a baseline creatinine of 84 μmol/L and eGFR of 71 mL/min. One month later, her creatinine was 167 μmol/L (eGFR 29 mL/min). A *de novo* anti-Class II HLA antibody was detected and acute antibody mediated rejection was diagnosed histologically. Despite treatment, her creatinine continued to rise and 2 weeks later was 403 μmol/L with an eGFR of 10 mL/min.

A kidney biopsy showed an extensive neutrophilic tubulitis with numerous fungal hyphae and spores. A fungal pyelonephritis and a perinephric collection both grew *C. albicans*. A renal biopsy showed tubular damage with a prominent mononuclear cell infiltrate, focal collections of neutrophils and numerous fungal spores. Her CRP was 46 mg/L. Blood and urine cultures also grew *C. albicans*. Fourteen days into antifungal drug treatment, a second renal biopsy showed fibrinoid necrosis with the interstitium markedly expanded by macrophages. A chest CT scan showed mycotic emboli in the lungs. After receiving 8.3 g of liposomal amphotericin B, she was changed to caspofungin (50 mg od) because her CRP remained elevated at 170 mg/L and her daily blood and urine cultures remained persistently positive (i.e. 22 positive sets taken on consecutive days) for *C. albicans*. Her CRP had risen to 227 mg/L.

IFN-γ immunotherapy was started. Five days later, an abdominal CT scan showed an increase in the size of the perinephric collection. It was drained. Culture of the pus

showed no growth, and her blood cultures (for the first time) were sterile at 5 days. Twenty days later, her neurological function deteriorated acutely. A CT scan showed that the perinephric collection was now much smaller in size. She then developed acute cerebral edema, tonsillar herniation and died. Her daily blood cultures became negative for *C. albicans* 5 days after starting IFN-γ and they remained sterile (i.e. 20 sets taken on consecutive days) until her sudden and unexpected death.

## Discussion

These uncontrolled observations in renal transplant patients with unpredictable fungal infection-related outcomes suggest that accelerated cure of high mortality, disseminated IFIs in kidney transplant patients is possible with the combination of IFN-γ immunotherapy and conventional antifungal drugs. Notably, a patient with disseminated (including cerebral) aspergillosis (mortality 90%) and a patient with disseminated (including cerebral) *O. gallopavum* infection (mortality previously 100%) were cured. The response during the 6-week period of treatment with a combination of antifungal drugs and exogenous IFN-γ injections was clinically dramatic. Furthermore, no patient has relapsed during long-term follow-up. This is the first patient case series report to suggest the potential benefit of adjuvant IFN-γ immunotherapy for accelerating the cure of life threatening IFI's infection in kidney transplant recipients.

Histopathology of the residual lesions after the completion of therapy showed the formation of tissue-based granulomas within 4 weeks of starting IFN-γ immunotherapy. Several of our patients developed histologically confirmed granulomas for the first time at the site of their infection within 4 weeks of starting IFN-γ immunotherapy. This was notable because granuloma formation is a Type IV delayed type hypersensitivity reaction in which Th1 cells mediate initial granuloma formation while other T-cell subsets promote its later phase development.

In most cases, it was also possible to continue the patients on their immunosuppressive drugs and maintain renal allograft function. During long-term follow-up, there was no clinical or laboratory-based evidence to suggest a detrimental effect of the IFN-γ injections on the kidney allograft. Many of the patients maintained stable renal function without any deterioration of their glomerular filtration rates (Table 1). This was despite the use of large cumulative doses of the potentially nephrotoxic drug liposomal amphotericin B (AmBisome) in all of the cases (31,32). No other clinical toxicity was seen except for IFN-γ-related fever and chills. This was easily managed with acetaminophen. It was notable that the occurrence of these symptoms was associated with clinical and microbiological evidence of rapid fungal pathogen clearance. These symptoms suggest that activation of therapeutic components of the innate immune response played a role in recovering from the IFI.

Our clinical findings are consistent with the emerging animal model-based conclusion that IFIs are associated with impaired phagocytic activity against fungi, high IL-10 levels, low IFN-γ levels and an inappropriate and often tissue destructive Th-17 host-mediated response (24,26–28,33,34). The inadequate Th1 host immune response (1,10,24,35–37) is compounded by the current use of T-cell directed transplant immunosuppressive drug regimens that also impair Th-1 T-cell immunity (10,35). Taken together, they could be contributing to the inadequacy of the IFN-γ-driven response to IFIs in some patients. In the cyclophosphamide-based neutropenic murine model of invasive aspergillosis, rapid and effective clearance of *A. fumigatus* in immunocompetent mice required an effective host Th1-orientated transcriptional program (24). Death was associated with a disordered transcriptional program and with increased expression of the genes encoding TNF-related proteins, TNF signaling and Th2/Th17 CD4 T-cell chemokines. The mice that died were also found to have a selective and severe deficiency of IFN-γ production within the infected lung itself (24).

Th1 responses are preferentially effective against intracellular pathogens and are primarily responsible for the production of the endogenous IFN-γ that plays such a major role in the effective eradication of cryptococci (38). However, Th1 responses are also actively involved in kidney allograft rejection (39) thereby explaining the targeted use of modern immunosuppressive drugs. For example, tacrolimus has been shown to inhibit intragraft mRNA expression of the Th1-cytokines IFNγ, IL-12 and TNF-α (40). In this context, a study in pediatric transplant recipients showed a significant reduction in IFN-γ production after patients were switched from CyA to tacrolimus (41). MMF also inhibits *de novo* purine synthesis and impairs T-cell immunity (42). Even low doses of steroids, when given in conjunction with calcineurin inhibitors, have been shown to suppress IFN-γ production (43).

As a T cell-derived macrophage activating lymphokine, IFN-γ is the most broad-acting antimicrobial and host-inducing cytokine that is currently available as an immunotherapeutic drug for clinical use. In the clinical setting, it promotes the antimicrobial activity of both blood monocytes and tissue macrophages (44) and has therefore been safely and successfully used to treat disseminated atypical mycobacterial infections (45) and drug resistant *Mycobacterium tuberculosis* (46). In the treatment of AIDS-related cryptococcal meningitis (in the pre highly active antiretroviral therapy era), there was also good evidence to show that the addition of IFN-γ to amphotericin B and flucytosine accelerated the time to mycological clearance and improved the clinical outcome (47,48).

Six of our seven patients had been treated for an episode of acute rejection prior to the development of an IFI. This highlights the important role of enhanced immunosuppression in increasing susceptibility to IFIs in these patients.

However, the time to infection was short in two cases (i.e. 1 and 7) at <1 month, and long in the other five cases (i.e. 9–96 months) (Table 2). In addition, only two patients (cases 4 and 5) received a dose of rituximab for acute antibody mediated rejection. This may be relevant in the context of the recent report of a substantial increase in both the incidence and mortality of IFI in kidney transplant patients treated with rituximab (49).

Our preliminary results suggest that IFN-γ immunotherapy could be safely used in combination with liposomal amphotericin B and other antifungal drugs in kidney allograft recipients who are at high risk of death from a disseminated invasive fungal infection. In the cases described, the clinical benefit of survival and cure of the invasive fungal infection far outweighed any potential risk of the allograft itself. Therefore, in the same way that the management of fungal infections in leukemic patients now demands an individualized treatment plan (19,20), so it becoming necessary to identify novel biomarkers in renal transplant patients that could enable the effective therapeutic use of adjuvant immunotherapies in well-defined patient subgroups. At the present time, large sums of money are spent giving prolonged courses of expensive antifungal drugs to these patients when they develop IFIs. In contrast, this 6-week course of IFN-γ injections cost $2600. The use of such a short course of combination therapy offers considerable potential therapeutic benefits in terms of:- (1) accelerated cure of refractory and life threatening invasive fungal infections; (2) no long-term relapse after stopping antifungal drugs; (3) saving of renal allografts; (4) no long-term deterioration in eGFR; (5) shortened hospital in-patient stay; (6) reduced morbidity and mortality; and (7) the considerable cost benefit of short-course antifungal drug therapy. Controlled clinical trials coupled to clinically useful new biomarkers that can reliably and reproducibly quantify the degree of immunosuppression are now required to define the unexpected efficacy of the therapeutic approach described.

## Conflict of Interest Statement

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