Surgical treatment of a foscavir-resistant atypical *Cytomegalovirus* pneumonia in an allogeneic stem cell transplant recipient

Pulmonary infections are a major cause of morbidity and mortality after allogeneic stem-cell transplantation (allo-SCT). Invasive aspergillosis is one of the major causes of pulmonary infection, but viruses are frequently involved, particularly human *Cytomegalovirus* (CMV).[1,2] Because of impaired cellular immunity, the risk for pulmonary infections is greatest during the early post engraftment period (from day 30 to 100). However, pulmonary infections also occur during the late phase (>100 days), and in particular in patients who developed acute or chronic graft versus host disease (GVHD) treated by steroids.[3] Pre-emptive strategies are now widely used for prevention of CMV disease in allo-SCT patients. Ganciclovir (GCV), and more recently valganciclovir (VGCV)[4,5] are the most commonly used drugs. However, late CMV disease still occurs, and prolonged antiviral therapies and/or recurrent viral reactivation episodes may lead to the emergence of resistant virus strains. Resistance mutations are located within the UL97 kinase and/or DNA polymerase (UL54) viral genes. In the absence of large prospective studies, the incidence of CMV resistance after allo-SCT is unknown. We report here a case of atypical CMV pneumonia after allo-SCT which was resistant to antiviral therapy and successfully treated by surgery.

Miss B. is a 64 year-old woman diagnosed with Ph positive chronic myeloid leukemia at a blastic phase in february 2005. A major molecular (2.10-5) response[6] was obtained after imatinib therapy and she underwent allo-SCT from an HLA phenoidentical unrelated donor in december 2005 (d0). The non-myeloablative conditioning regimen included total body irradiation and fludarabine.[7] She received antithymocyte globulin, cyclosporine, and mycophenolate mofetil for graft versus host disease (GVHD) prophylaxis. She was also given valaciclovir (VACV, 3 g/d) for the prevention of herpes simplex virus infections. Both patient and donor were CMV seropositive. CMV DNAemia was monitored by *in house* real time quantitative PCR (adapted from ref. #8). At d28, she developed histologically confirmed grade III-IV9 GVHD. She was successfully treated with steroids. At d45, a first CMV reactivation occurred and was pre-emptively treated by sequential GCV/VGCV therapy (Figure 1). CMV viral load (VL) became negative at d76. At d90, while still under steroids (2 mg/kg), she presented cutaneous grade III GVHD treated by PUVA therapy, in association with the other immunosuppressors (mycophenolate mofetil and ciclosporine). At d180, while she was under VACV, she presented with hyperthermia, thrombosis and pulmonary embolism, associated with high CMV VL (4.4 $\log_{10}$cop/10^6 peripheral blood leukocytes [PBL]). In addition to anti thrombotic therapy, she received VGCV at curative dose for 14 days, followed by maintenance therapy. Low viral replication (<3 $\log_{10}$cop/10^6 PBL) persisted under VGCV, and a GCV-resistant strain was detected by UL97 genotyping in blood at d220. The resistance mutation was retrospectively detected in blood from d180 (Table 1). Because of diarrhea (d229), biopsies were performed and histological examination revealed CMV colitis. Alternative therapy with foscavir was initiated and permitted a significant decrease of CMV DNAemia (<2,5 $\log_{10}$cop/10^6 PBL) associated with partial regression of colitis symptoms. From d200, Miss B presented a chronic hacking cough. The first two computed tomography (CT) scans at d238 and 250 did not show any distinctive lesions. Pulmonary aspergillosis was suspected on the third CT at d292, that showed a unique pulmonary mass located in the upper right lobe (Figure 1). Aspergillosis antigenemia and bronchoalveolar lavage remained negative. Nevertheless she

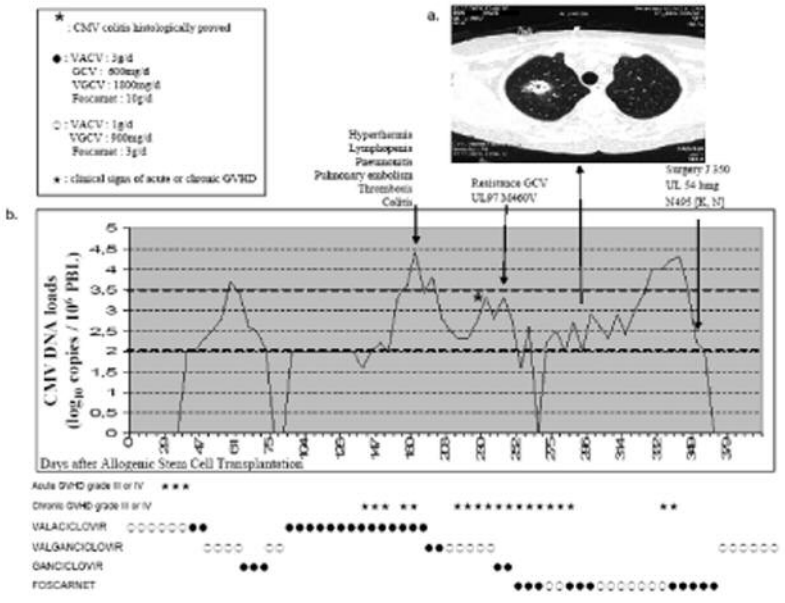

Figure 1. (A) CMV DNA loads were monitored by *in house* real time quantitative PCR, performed after whole blood automated extraction. Viral gene was coamplified with human albumin gene by duplex real time PCR. Viral loads were normalized to the number of cells resulting from the albumin gene quantification and expressed as CMV genome copies/10^6 peripheral blood leucocytes. Under 2 $\log_{10}$cop/10^6 PBL, VL were considered negative. Preemptive treatment was initiated at VL >3.5 $\log_{10}$cop/106 PBL (dotted lines). Antiviral treatments, with respective doses, are presented under the graph. (B). Pulmonary CT, performed on day 292, was highly evocative of aspergillosis, because of the presence of the "halo" sign around the pulmonary mass, located in the upper right lobe.

Table 1. UL 97 (from nucleotide 1341 to 1990) and UL54 (from nucleotide 870 to nucleotide 3180) amplified products, recovering all known resistance mutations, were submitted to direct sequencing using the ABI Prism Big Dye Terminator Cycle sequencing kit (Applied Biosystem).

day after transplant	source	UL97 resistance mutations	UL54 resistance mutations	UL54 polymorphisms
180	blood	**M460[M,V]**	—	—
202	blood	**M460V**		
229	blood	**M460V**	—	—
271	blood	—	none	N685S A885T N898D L948M*
291	blood	none	none	N685S A885T N898D V946 [V,F] *
324	blood	none	none	N685S A885T N898D V946 [V,F] *
345	Lung biopsy	none	**N495[K,N]**	N685S A885T N898D L948[M,L]* V946 [V,F] *

Sequence analysis was performed using SeqScape Software vs 2.5. Genotypic analysis of UL54 mutations showed double populations at the same locus, and illustratesing the presence of multiple strains in the same biological sample. Three polymorphisms (N685S, A885T, N898D) were observed in all the samples analysed (d271, d291, and d324 post allograft). One polymorphism (L948M) was detected in the d271 blood sample and in the lung biopsy but not in the two other blood samples. One other polymorphism (V946F) was observed in d291 and d324 blood samples and in lung. Finally, the strain harbouring the foscarnet resistance mutation (N495K) was detected only in lung.

received antifungal therapies including voriconazole, caspofungin, liposomial amphotericin B and posaconazole. Low level CMV replication was sometimes detected under foscavir maintenance therapy, from d254 to d310. From day 320, CMV VL increased again to >3.5 logcop/10^6 PBL. She was again given foscavir (10 g/d), allowing rapid decrease of CMV replication that fell to <2.5 $\log_{10}$cop/10^6 PBL at d340. Because of clinical aggravation, increase of the pulmonary mass (despite antifungal treatment) and a major hemorrhagic risk, a surgical lung resection was performed at d345 (*T. Gastinne, written communication, American Society of Hematology 48th Annual Meeting*). Unexpectedly, histological diagnosis showed local tissuer destruction associated with fibrosis and a polymorphic inflammatory infiltrate. Around this lesion, dystrophic pneumocytes were observed, characterized by hypereosinophilic cytoplasma and enlarged nuclei with inclusion bodies. Anti CMV immunohistochemistry staining was positive on these pneumocytes. No other opportunistic bacterial or fungal infection could be found, including aspergillosis. UL54 genotyping on sequential blood samples and a pulmonary resection fragment showed the presence of several virus strains at the time of pulmonary resection: at least two different CMV strains were present in blood, detected by a double population at position 946 (V/F). They were also detected in the lung, in addition to two other double populations at position 948 (V/F) and 495 (N/K), the last one conferring resistance to foscavir (Table 1). Finally, from d352 to seven months after surgical exeresis, the patient was still negative for CMV DNAemia.

The predominant patterns of abnormality on high resolution CT of CMV pneumonia are usually ground-glass opacities, small centrilobular nodules and air-space opacities.[10] This case thus reports a very unusual clinical presentation of a CMV pneumonitis. A unique mass with halo sign was detected on CT, which is an early sign highly evocative of pulmonary aspergillosis.[11,12] This clinical presentation underlines the possible difficulty of CMV disease diagnosis, the importance of histological confirmation of viral or fungal infection, and of the retention of local samples for the isolation of CMV strains. It also illustrates the emergence of resistance mutations against a history of CMV infection during the months following SCT. The M460V mutation, frequently associated with GCV resistance, has been described after pre-emptive VGCV therapy in SCT.[13,14] However, its early emergence under VACV after only 30 days of VGCV/GCV cumulative therapy was surprising. It was certainly favoured by persistent viral replication under VACV selection pressure, as reported *in vitro*.[15] Early emergence of M460 mutation has been reported only twice in patients who received VACV or ACV prior to GCV as prophylaxis.[16,17]

Genotypic analysis of UL54 polymorphism showed double populations at the same locus and illustrated the possible presence of multiple strains in the same biological sample. The N495K foscarnet resistance mutation, previously reported only once,[18] was detected only in the lung. As the resistant strain was not detected in the blood compartment, and in the absence of early pulmonary samples, the chronology of the emergence of the resistant strain is difficult to determine. A double population at position 948 (L948M) was detected in blood at d271 and then in the lung resection fragment, but not in blood at d324. A double population at position 946 was detected in blood and in lung at d324. We can thus hypothesize that the strain harbouring polymorphism L948M also harboured the resistance mutation N495K. This observation illustrates the possible compartmentalization of CMV strains, that has been infrequently reported in the setting of SCT.[13,19,20] Direct genotypic analysis of CMV strains isolated in blood may not always predict the resistance profile in the tissue. Once again, emergence of the resistant strain had been favoured by continuous low rate viral replication under drug pressure. The foscarnet resistance mutation emerged in a limited pulmonary lesion, and we hypothesize that tissue penetration of foscarnet was not sufficient to control viral replication. This observation underlines the importance of using curative doses and avoid-

ing prolonged low dose maintenance therapy. Moreover, it illustrates the need to monitor for resistance when rising viral loads or persistent shedding occur after several weeks to months of therapy.

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