How to best manage cytomegalovirus infection in the allogeneic stem cell transplant setting: future prospects

Carlos Solano^{1,2} & David Navarro^{*,3,4}

“The use of antivirals with relatively high intrinsic activity against cytomegalovirus ... has dramatically decreased the incidence of early cytomegalovirus disease...”

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Allogeneic stem cell transplantation (allo-SCT) was systematically adopted in clinical practice in the early 70s of the past century, and it soon became the sole curative procedure for a number of hematological malignancies refractory to conventional chemotherapy treatment regimens. In these early days, cytomegalovirus (CMV) infection emerged as a dreadful threat for allo-SCT patients, more than a third of them developing end-organ disease, most frequently pneumonitis or gastrointestinal disease, whose treatment was often unsuccessful [1]. The use of antivirals with relatively high intrinsic activity against CMV, such as (val)ganciclovir or foscarnet, administered either prophylactically or pre-emptively, has dramatically decreased the incidence of early CMV disease (occurring within the first 100 days

after transplant), which is currently diagnosed in less than 5% of allo-SCT recipients [1,2]. Universal prophylaxis, consisting of the administration of antivirals to all patients, usually at the engraftment, irrespective of their particular risk of CMV disease, leads invariably to overtreatment, as many patients who would remain free of CMV disease are nevertheless treated. In addition, development of late-onset disease (occurring after day 100 after transplant), once the antiviral is discontinued, most likely as a result of a suboptimal reconstitution of CMV-specific T-cell immunity, is a non-negligible drawback [1]. Moreover, drug-related toxicity (hematological, most notably) is a major side effect of this approach [1]. Because of all the above, the use of this strategy is restricted to high-risk patients and only applied in very few

KEYWORDS

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¹Hematology Service, Hospital Clínico Universitario, Instituto de Investigación INCLIVA, Valencia, Spain

²Department of Medicine, School of Medicine, University of Valencia, Spain

³Microbiology Service, Hospital Clínico Universitario, Instituto de Investigación INCLIVA, Valencia, Spain

⁴Department of Microbiology, School of Medicine, University of Valencia, Spain

*Author for correspondence: Tel.: +34 96 386 4657; Fax: +34 96 386 4173; david.navarro@uv.es

“Functional cytomegalovirus-specific T cells are essential in the prevention and resolution of viremic episodes.”

centers. The advent of new antivirals with an increased intrinsic activity against CMV, and a safer toxicity profile, such as maribavir, brincidofovir and specially letermovir, has renewed the interest in universal prophylaxis [1]. Yet these drugs are not totally harmless and as it has been recently shown, viral resistance may emerge during treatments, a potential event that we cannot afford [3]. In this setting, a reasonable goal would be to administer antiviral prophylaxis exclusively to those patients carrying a truly increased risk of viremia (targeted prophylaxis). Unfortunately, pre- and post-transplant clinical factors (i.e., type of transplant, donor/recipient CMV-serostatus, occurrence of severe acute graft vs host disease), known to modulate that risk, are insufficiently predictive. We hypothesized that this approach may be feasible by combining clinical, genetic, biological and immunological data of patients to build a predictive risk score [1]. Genetic traits predisposing to the development of CMV viremia are being increasingly reported. In fact, donor or recipient single nucleotide polymorphisms (SNPs) located within genes (or close to them) regulating innate and adaptive immune responses to CMV, such as Toll-like receptor 2 (TLR-2), chemokine receptor-5 (CCR-5), monocyte chemoattractant protein-1 (MCP-1) or interferon $\lambda 3$ (IL28B) have been shown to impact on the risk and the dynamics of CMV viremia [1]. Large studies proving this assumption are nevertheless lacking. Moreover, sequential measurement of plasma levels of pro-inflammatory cytokines, prospective metabolomic profiling of plasma specimens and monitoring of CMV-specific CD4⁺ and CD8⁺ T-cell responses by the use of HLA-peptide multimers, flow cytometry for cytokine staining, ELISPOT or by the commercially-available Quantiferon CMV assay were shown to allow anticipation of the occurrence of viremia with reasonable good positive predictive values (see [1] for a review). Naturally, much more work needs to be done in this area, but preliminary data warrants further investigations.

Pre-emptive antiviral therapy, consisting of the inception of antiviral treatment upon detection of viremia is currently the standard of care for the prevention of CMV disease in allo-SCT recipients [1,4]. At most transplant centers, monitoring of CMV DNA load either in plasma or whole blood by real-time quantitative PCR assays (QRT-PCRs) is performed to make decisions as to when to start and when

to stop antiviral treatment. The CMV DNA load cutoff prompting the initiation of antiviral therapy varies widely across centers [1–2,4]. Most transplant programs use a pre-established CMV DNA load threshold, irrespective of the patient clinical risk of CMV disease (between 500 and 1500 IU/ml for plasma or between 1000 and 10,000 IU/ml for whole blood). Other centers follow a risk-adapted strategy, starting antiviral therapy upon plasma CMV DNA loads as low as 100–150 IU/ml in high-risk patients [4]. Likewise, antiviral therapy is interrupted upon the first undetectable viral load result in some centers or after two consecutive negative PCR results in others [1,4]. There is little doubt that achieving consensus criteria for triggering and interrupting pre-emptive antiviral therapy would be beneficial for patients. This has not been possible because a straight comparison of experiences across centers is not feasible due to the exceeding inter-assay and inter-laboratory coefficients of variation of QRT-PCR assays and the variable efficiency of DNA extraction methods [5,6].

The advent of international standards for CMV DNA load quantitation, in particular the WHO International Standard from the National Institute of Biological Standards and Controls [7], which allows normalization of CMV DNA load values between assays, now reported in IU/ml, raised great expectations. Nevertheless, the commutability of these standards is suboptimal [8], and as it could be anticipated, significant inter-assay and inter-center variations for individual specimens persist [9].

The limit of detection of CMV QRT-PCR assays has been lowered dramatically in recent years (20–35 IU/ml, for some of them). In this context, a reasonable question is why withhold antiviral treatment until a given CMV DNA load in blood is reached? A reasonable answer is that by doing so, antiviral treatment can be spared to almost 50% of viremic patients who would be capable of controlling CMV replication upon a prompt activation and expansion of functional virus-specific T cells, thus avoiding drug-related toxicity. This strategy assumes that the presence of CMV in the blood compartment is permissible as long as it does not reach a level that would put the patient at an imminent risk of overt CMV disease (>1000–1500 IU/ml). This view, nevertheless, has been recently challenged by Green *et al.* [10] who reported that CMV DNA loads as low as 250 IU/ml, reached upon the first

100 days after transplant, are associated with an increased risk of non-relapse death caused by infectious complications, a phenomenon presumably related to the immunosuppressive properties of CMV (indirect effects). These data are difficult to reconcile with earlier findings demonstrating that prophylactic administration of ganciclovir at engraftment and delaying the start of ganciclovir until high-grade antigenemia resulted in similar survival rates [11], and thus must be confirmed in multicenter prospective studies. Likewise, the idea that early initiation of pre-emptive therapy (at viral loads as low as 135 IU/ml) may result in shorter courses of antiviral therapy [12] merits further research. Until then, to limit drug overexposure and thus toxicity, the focus must be placed on treating as few patients as possible, without compromising their safety, and using antiviral treatment courses as short as possible, without increasing the patient's risk of relapsing/recurrent episodes and late end-organ disease. We believe that kinetic analyses of plasma CMV DNA load and routine monitoring of CMV-specific T-cell responses may help to achieve these purposes. In effect, we recently showed that a CMV DNA load doubling time (dt) ≤ 2 days, anticipated the eventual need of preemptive antiviral with a positive predictive value of 100% [13]. We later reported that treating patients upon $dt_s \leq 2$ days led to a significant reduction of the time on antiviral therapy [14]. In addition, we proved [15] that the CMV DNA dt may be a valuable parameter for inter-assay and inter-institutional results comparison since QRT-PCR assays show linearity above their limit of quantification, with slope coefficients that vary minimally when testing standard calibration panels [9].

Functional CMV-specific T cells are essential in the prevention and resolution of viremic episodes (reviewed in [1]). Sequential monitoring of CMV-specific T-cell responses has been shown to identify those patients of whom antiviral treatment can be spared safely at the time of CMV viremia detection [16], and may help to tailor individually the length of antiviral therapy courses. Previous work by our group showed that patients treated preemptively with antivirals and

expanding CMV-specific phosphoprotein (pp) 65 and immediate-early (IE)-1 interferon (IFN)- γ CD8⁺ T cells at levels >1 cell/ μ l at the time of CMV DNAemia clearance had a reduced risk of developing recurrent episodes of viremia, even using shorter courses of antiviral therapy [17]. We recently conducted a multicenter, closely-matched comparison-group study in which this assumption was found to be correct for a subset of patients [18]. Surely, the near future will witness the resurgence of the debate on whether universal prophylaxis with the new anti-CMV drugs or pre-emptive antiviral therapy must be the standard of care for the prevention of CMV-related clinical complications in allo-SCT recipients. In the meantime, research efforts should be concentrated on identifying precisely which patients are at high risk of developing uncontrolled viremia (tributary of targeted prophylaxis), determining whether low-level viremia has a tangible detrimental effect on patient survival, reducing the inter-assay variability of QRT-PCR assays, evaluating the validity of the dt for inter-center comparisons, and determining to what extent routine immunological monitoring may contribute to improving the management of CMV infection in these patients by conducting well-designed randomized controlled clinical trials.

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