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Reply to: "Time is a crucial factor for the use of oncological treatment for post-transplantation recurrence of hepatocellular carcinoma"

To the Editor:
We thank Dr. Felga and colleagues for their comments on our article demonstrating the efficacy of sorafenib treatment in case of recurrent hepatocellular carcinoma (HCC) after liver transplantation (LT) [1]. In their experience with 20 of such patients in Brazil they observed that time-to-recurrence (TTR) after LT might be influenced by adverse baseline tumor biology – i.e., G3 tumors and presence of macrovascular invasion – and tumor load, suggesting that early recurrences within 1 year after LT were characterized by a worse prognosis, while patients with later recurrences were more prone to receive some form of HCC treatment and had a higher median survival.

Indeed, we thank for the opportunity to add to our previous report a few comments on the supposed differences among early *vs.* late recurrences after LT. Differently from Dr. Felga *et al.*, we did not find that HCC factors such as tumor load (within/beyond Milan criteria at radiology and pathology), presence of microvascular invasion (mVI) and presence of microsatellites were correlated with TTR (with $p = 0.217$, 0.510, 0.128, and 0.510 respectively at the Pearson Chi-square test). Only a higher number of nodules *per se* – evaluated as a continuous variable at pre-LT radiology and at histology – appeared to be associated with an earlier recurrence ($p = 0.019$ and 0.036 respectively at the Kruskal-Wallis test). Therefore, even if the aforementioned HCC characteristics are associated with the probability of tumor recurrence after LT [2], they are apparently poor predictors of the TTR after LT.

Although TTR is not predictable, timing and pattern of the observed recurrences may happen to be correlated. In our series, patients with early recurrence suffered that in multiple organs more frequently than patients with a later recurrence (63.6% *vs.* 28.6%, $p = 0.43$). This resulted in a reduced eligibility to curative treatments – such as surgical resection or radiofrequency ablation – that were applied only in 18.2% of those with early recurrence *vs.* 75% of late recurring patients ($p = 0.001$), since also the median time to untreatable progression/presentation was significantly different among groups [0.5 months (0.5–54.8) *vs.* 11.1 months (0.5–77.4), $p = 0.131$ respectively]. As brilliantly stated by Toso *et al.* [3], early recurrences may be linked to remaining extrahepatic HCCs deposits left at the time of LT, or result from the post-transplant engraftment of aggressive circulating HCC clones. Such a tumor aggressiveness, that influences treatment applicability and patients outcome, has been previously demonstrated by different authors [4,5] and confirmed herein by Felga *et al.*

In our recent paper, we observed that the more indolent course of late HCC recurrences, occurring more than 2 years after LT, is associated with a reduction in the risk of death of more than 70% if compared to early recurrent patients. Noteworthy, the median TTR of those 5 patients of our series remaining alive and cured after surgical resection of their recurring HCC approached 5 years (58.7 months). In these patients HCC recurrence is probably the result of the reactivation of a few dormant HCC cells, engrafted at the time of LT: in such circumstances a prompt surgical removal may be associated with some chance of cure. Conversely, when facing early recurrences after LT, an aggressive surgical treatment may be useless and even harmful considering the high risk of further growth of occult disease. As suggested [3], the first step in the treatment of an early intrahepatic recurrence may be a loco-regional treatment, used as a test of time in order to observe potential progression in other sites. In the instance of an early recurrence emerging in multiple organs, or when facing an untreatable progression, we recommend the use of sorafenib at the onset of recurrence, considering its safety and possible benefit in survival [1,6].

Conflict of interest

The authors have received lecture fees from Bayer Healthcare for training courses with educational purposes on hepatocellular carcinoma.

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Controversy on the role of FoxP3$^+$ regulatory T cells in fibrogenesis in chronic hepatitis C virus infections

To the Editor:

We read with interest the manuscript by Langhans and colleagues. The authors identified a subset of intrahepatic FoxP3$^+$ regulatory T cells (Treg) that produce IL-8 in chronic hepatitis C virus (HCV) infected patients, and the authors suggested that these cells promote liver fibrosis through stimulation of hepatic stellate cells [1]. This article is very timely and highly relevant in an age where novel therapeutic strategies based on the use of direct-acting antivirals are expected to lead to viral eradication in most chronic HCV patients who undergo treatment. However, despite dramatically improved viral eradication rates, the detrimental consequences of liver pathology and fibrosis, such as decompensated liver cirrhosis and hepatocellular carcinoma, remain an issue, and detailed understanding of fibrosis is needed.

The paper by Langhans *et al.* makes an important contribution to better understand the role of Treg in fibrosis in chronic HCV by showing that IL-8-secreting Treg are enriched in the liver and are detected especially at the transition areas of fibrosis. Although this is a very intriguing observation that identifies a functionally specialized fraction within the Treg pool of cells, it also raises a number of questions. First of all, only a minority of liver FoxP3$^+$ Treg produce IL-8 (on average 4.5%), while it is known that Kupffer cells, but also hepatocytes, endothelial cells, epithelial cells and fibroblasts are major sources of IL-8. The paper does not clarify what determines that Treg-derived IL-8 promotes fibrinogenesis, whereas the importance of IL-8 derived from other liver cells is still unknown. Importantly, the IL-8 levels secreted by these cells are likely magnitudes higher. Next, an interesting finding from the microarray analysis presented by the authors is that Treg clones generated from cells from chronic HCV patients also express IFN-γ, besides IL-8. Both cytokines are expressed at lower levels by clones from patients with spontaneously resolved HCV infections. It would be relevant to examine whether the IL-8/IFN-γ producing Treg clones also possess effector activities that are independent of IL-8 and may promote fibrosis. In this respect it is important to mention that a general feature of tissue-residing T cells is an activated phenotype [2], which may be more pronounced in livers from persistent *vs.* resolved patients. Finally, IFN-γ producing capacity is generally not a feature of FoxP3$^+$ Treg, which raises the question whether the Treg clones described by Langhans *et al.,* are representative for intrahepatic FoxP3$^+$ Treg *in vivo*.

In apparent contradiction with the population of IL-8 secreting Treg described by Langhans *et al.*, we recently published data in the *Journal of Hepatology* in line with the widely accepted paradigm that FoxP3$^+$ Treg limit immunopathology at the cost of insufficient protective pathogen-specific immune responses [3,4]. Our findings showed that FoxP3$^+$ Treg were abundantly present in chronic HCV infected livers, but not in healthy livers, and primarily resided within the portal tracts in close proximity to the lymphocytic infiltrate, which is in line with previous findings published in the *Journal of Hepatology* by two independent groups [5,6]. Additionally, in our hands, higher frequencies of FoxP3$^+$ Treg were present in livers of chronic HCV patients without or with mild fibrosis [4]. Although Langhans and colleagues kindly referred to our publication, they omitted to discuss these key findings.

If it holds true that IL-8 production by FoxP3$^+$ Treg substantially contributes to liver fibrosis, we propose that, analogous to TGF-β [7–9], FoxP3$^+$ Treg play a dual role both promoting and limiting fibrosis. While FoxP3$^+$ Treg generally seem to protect against liver fibrosis [4], the small subset of IL-8-expressing FoxP3$^+$ Treg may deteriorate liver pathology [1]. Importantly, in contrast to what is often postulated, we recently showed that FoxP3$^+$ Treg are retained in the liver long after HCV eradication, even after HCV RNA was undetectable in serum for more than 12 months, suggesting ongoing immunopathology and regulation by intrahepatic FoxP3$^+$ Treg [10]. These FoxP3$^+$ Treg differed phenotypically from their counterparts present during ongoing persistent infection. We strongly feel that these Treg are unlikely to further promote fibrosis in the absence of HCV, but rather act as memory Treg. The question remains whether IL-8 production by FoxP3$^+$ Treg is maintained after viral eradication, or whether IL-8 expression is reduced. Our data as well as Langhans' findings demonstrate convincingly that the Treg compartment in HCV infected livers is heterogeneous, and more detailed analysis, both phenotypically as well as functionally is urgently needed to elucidate the importance of FoxP3$^+$ Treg in disease progression in the inflamed liver.

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