Reply to "*Response to* "CD20 positive cells are undetectable in the majority of multiple myeloma cell lines and are not associated with a cancer stem cell phenotype". Haematologica 2012;97(7):1110-1114

We read with interest the letter by Van Hoef[1] about the article by Paino *et al*.[2] In that study, we were unable to identify a CD20$^+$ population among 8 myeloma cell lines leading us to conclude that CD20 may not be a suitable antigen for the identification of myeloma cancer stem cells.[2] These results contradict early observations in the 1990s by Pilarsky[3] and in the early 2000s by Matsui and colleagues.[4] In turn, recent data point to a more prominent role of plasma cells (PCs), rather than B cells, in the pathogenesis of this disease.[5]

Studies in multiple myeloma (MM) were some of the first to unravel the potential presence of so-called cancer stem cells (CSCs), and this area of research is more active than ever. The introduction of high-dose therapy and novel agents has improved complete response rates and doubled MM patients' survival,[6] but unfortunately the majority of patients relapse as only a minority is considered in *operational cure*; this might be attributable to the presence of a MM-CSC. This cell compartment would fit into the classical definition of CSCs: a small number of cells with high tumorigenic potential through the process of self-renewal.

The letter by Van Hoef raises the question of the utility of cell lines for the study of the stem cell in myeloma. Although aware of the limitations of cell lines, it should be taken into account that in tumors such as breast cancer, the characterization of cancer stem cells is much more advanced than in myeloma, in part thanks to the results obtained with cell lines.[7] This prompted us to use myeloma cell lines as a tool to study the myeloma stem cell.

We agree with Van Hoef that it is likely that myeloma develops out of PCs in the bone marrow, and not out of cells in the lymphoid tissue; however, we disagree that PCs do not circulate in the peripheral blood, unless after stimulation. We and others have shown that both normal and myeloma circulating PCs can be naturally found in the peripheral blood of normal individuals[8] and patients with a monoclonal gammopathy.[9] Although the latter are present in extremely low numbers (typically <0.1%), they are a valuable marker of malignant transformation in monoclonal gammopathy of undetermined significance (MGUS)[10] and smoldering MM,[11] as well as of disease aggressiveness in symptomatic MM.[12] In fact, patients at the latest stage of the disease may often show the presence of a PC leukemia. Accordingly, one may ask whether, in the context of disease dissemination and metastasis, these cells represent specific subclones with higher propensity to circulate and home to new bone marrow niches, or if they harbor stem cell-like features. These questions remain to be answered.

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