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Authors' Reply

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We thank Dr. Küçük and colleagues for their comments on our published article¹ and for raising their concerns about our conclusion that *HACE1* is not a tumor suppressor gene in natural killer/T-cell lymphoma (NKTCL).¹

The first criticism refers to the fact that we mainly studied T cell lines in which the *HACE1* tumor suppressor gene in NKTCL was reported in NK-cell lymphoma. NKTCL is essentially a disease of cytotoxic cells in which the nature of T or NK cells has never been related to the differences in clinical presentation, treatment response, or prognosis, although about 80% of NKTCL cases have been reported to be of NK origin. We studied six NKTCL cell lines of which only half were of T cell origin. We also studied three NK cell lines: MECO4,² YT, and SNK6. SNK6, in particular, harbors the 6q21 deletion with no difference in the T and NK cell lines concerning the lack of apoptosis induction by *HACE1*.

The second criticism concerned the use of IL-2-activated peripheral blood lymphocytes (PBLs) as control for protein detection. NKTCLs are not exclusively of NK origin; we were therefore careful to have a control population to reflect both T- and NK-cell content. Indeed, with high-dose IL-2 present from the beginning of the PBL culture—circulating NK cells constitutively express a functional IL-2 receptor comprising the noncovalently associated CD25, CD122, and CD132—and after a short time of amplification (7 to 10 days), we obtained a PBL–IL-2 cell population that contained 20% to 30% NK cells. We believe that such a cell population is a relevant control for NKTCL cell lines.

Third, regarding the experiments presented in Figure 5 and use of 7AAD versus Annexin V to measure apoptosis: we disagree that 7AAD measures only late apoptotic cells. The

use of 7AAD to measure apoptosis is well supported. In addition several publications demonstrate that 7AAD detects the loss of cell membrane integrity, an early event in cell death, and therefore measures early (7AAD^{lo}) as well as late (7AAD^{hi}) apoptotic cells with similar results in direct comparison with Annexin V staining.^{3–5}

Finally, concerning the pertinence of TAT fusion protein for *HACE1* expression strategy, we presented convincing evidence that TAT-*HACE1* penetrates the cells and is functional once in the cytoplasm. This strategy required a very short incubation (10 minutes) of the target cells with recombinant protein but without selection, as almost all cells internalized TAT-*HACE1*. In fact, the data presented in Figure 4B show the colocalization of TAT-*HACE1* with the Golgi marker GM130.¹ The low amount of overlap may be explained by the fact that *HACE1* does not interact directly with GM130; its ligands are Rab1 and Rab4, but their expression is not limited to the Golgi. TAT recombinant proteins have been used widely with success, particularly to overexpress proteins with post-translational modifications such as phosphorylation. The data in Figure 4 also show that TAT-*HACE1* expressed in the NKTCL cell lines is functional and capable of ubiquitinating RAC1-GTP.

We therefore are convinced that our results¹ strongly argue against an essential role of *HACE1* as tumor suppressor gene in NKTCL. It is also important to remember that *HACE1* is always expressed in NKTCL and that a loss of function mutation has yet to be reported in these patients.

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