Natural killer (NK) cells are members of the innate immune system with potent effector function against tumors. Their ability to kill transformed cells directly without prior sensitisation, combined with a lower risk to induce graft-versus-host disease make them attractive candidates for cell-based immunotherapy. However, there are some limitations to such therapies, associated with limited in-vivo persistence and function of NK cells depending on the tumor microenvironment. In contrast to hematological malignances, only a limited anti-tumor effect is observed following NK cell transfer in patients with solid tumors. This is likely due to impaired NK cell infiltration, survival and performance within the hypoxic tumor microenvironment. Hypoxia is a common aspect of most solid tumors; it alters cancer and immune cell metabolism, leading to metastasis formation and resistance to therapies.

A better understanding of the role of hypoxia in NK cell activation would allow to increase the effector function on these cells and to optimise their implementation in targeting hypoxic tumors.

Hypoxia induces the hypoxia-inducible factor (HIF) pathway, and we have previously shown that NK-cell-specific deletion of HIF-1a leads to reduced NK cell counts in the spleen and to impaired NK cell activation and effector function in vivo and in vitro. In a gain of function model, we now show that loss of Von Hippel–Lindau (VHL) protein in NK cells leads to improved survival of NK cells, both, in normoxic and hypoxic conditions. Noteworthy, whereas wildtype NK cells require IL-15 for survival and proliferation, VHL-deficient cells show enhanced survival and proliferation even without IL-15 stimulation. This phenotype is no longer observed in HIF-1a-VHL KO NK cells, suggesting that the gain of function in VHL KO cells depends on HIF-1a function. VHL deletion also affects NK cell cytotoxicity: in response to stimulation with cytokine combinations (IL-2, IL-12, IL-15 and IL-18) VHL-deficient NK cells show an enhanced expression of cytotoxic FasL and higher IFNγ production. Also, cells stimulated with ligand activation of the NK1.1 and NKp46 receptors present a higher degranulation capacity. Thus, boosting the hypoxic response in NK cells leads to enhanced expansion as well as improvement of specific functional readouts.

These findings suggest the potential use prolyl-hydroxylase inhibitors to boost NK cell expansion and performance in the adoptive transfer setting.

**SPECIAL TECHNIQUES AND EQUIPMENT**
Flow cytometry, in vivo studies, cell culture, cytotoxic assays