of T-cells into a regulatory phenotype. In a murine B16 xenograft model IL-2 expression significantly enhanced therapeutic effects of an H1 oncolytic influenza virus. Expressed within the background of H5 hemaggutinin, IL-2 did not lead to a significant enhancement of therapeutic efficacy. Interestingly, the empty influenza H5 subtype was significantly more potent in treating B16 xenograft tumors than the H1 subtype, regardless of IL-2 expression. In primary human PBMC models, the virus based on H1 hemaggutinin led to enhanced CD8 T-cell activation compared to H5. This effect was further enhanced by IL-2 expression, although all viruses led to significant activation. Surprisingly, viruses based on H1 hemaggutinin led to increased expression of the immune checkpoint PD-1. The virus based on H5 hemaggutinin did not lead to upregulation of PD-1, indicating a favorable balance between activation and exhaustion. Infection with the H5 based virus led to both enhanced apoptosis and immunogenic calreticulin exposure in human and murine melanoma cell lines compared to H1.

Conclusions IL-2 does not promote T-reggs, when expressed in a viral background. H1 viruses induced PD-1 more potently than H5 viruses. The choice of viral entry protein is more relevant for the therapeutic effect of the virus, than the expression of a T-cell stimulating cytokine such as IL-2. Efficacy of oncolytic viral treatment appears to depend more on viral growth than on virally expressed T-cell promoting cargo.