The HDAC6-specific inhibitor AVS100 (SS208) blocks M2 polarization of tumor associated macrophages and potentiates immunotherapy in preclinical tumor models

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Abstract

Inhibition of HDAC6 was associated with an increased proinflammatory tumor microenvironment and an antitumoral response. Here, we show that a highly specific HDAC6 inhibitor AVS100 (SS208), blocks M2 polarization in murine and human macrophages while partially affecting M1 polarization. AVS100 effects were observed as blocked upregulation of M2-related gene signature under M2 polarizing conditions and blocked generation of CD206+ and Arg1+ macrophages. Oral administration of AVS100 had an antitumoral effect in SM1 melanoma and CT26 colon cancer models and increased the efficacy of anti-PD1 treatment, leading to complete remission in melanoma and increased response in colon cancer. Flow cytometry and scRNAseq analysis of tumor-infiltrating immune cells revealed an increase of proinflammatory/anti-inflammatory ratio in tumor-associated macrophages as well as an increase of intratumoral CD8 effector T-cells after AVS100 treatment. Interestingly, cured mice didn't relapse and became resistant to a subsequent tumor challenge, suggesting acquired antitumoral T-cell immunity. T-cell repertoire analysis of effector/memory T-cells in cured mice revealed a higher number of immunodominant T-cell clones after AVS100 treatment, indicating increased T-cell expansion.
Finally, AVS100 has demonstrated no mutagenicity and a strong safety profile in rats and dogs, leading to its recent U.S. FDA clearance of an Investigational New Drug (IND) application and planned initiation of Phase Ia/b clinical trials targeting locally advanced or metastatic solid tumors in the first half of 2024.
Altogether, we have performed the preclinical characterization of a novel small molecule inhibitor targeting HDAC6 for solid cancers. AVS100 had an antitumoral effect as single agent and improved the efficacy of immune checkpoint inhibition by blocking the immunoregulatory tumor microenvironment and increasing T-cell immunity.

Conclusions

We show that inhibition of HDAC6 by AVS100, reduces c-Myc levels and blocks the M2 differentiation of murine and human macrophages. This effect changed the tumor microenvironment to increase pro-inflammatory and reduce anti-inflammatory macrophages leading to an increased effector T-cell signature and an anti-tumoral effect. AVS100 increased the amplification of immunodominant intratumoral T-cell clones and improved the efficacy of immunotherapy.

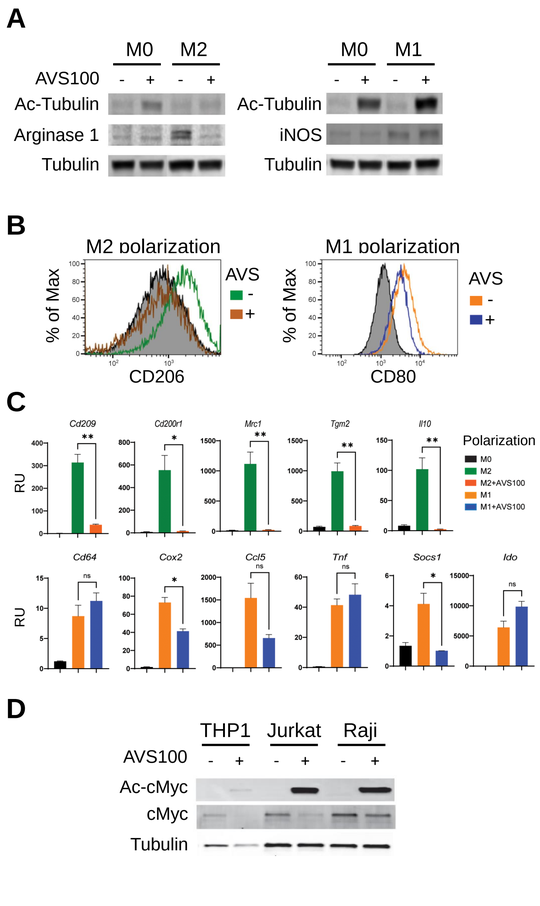

Figure 1. AVS100 blocks the M2 polarization of macrophages

A) Western-blot showing that AVS100 treatment prevented the induction of the M2 marker Arginase 1 and the M1 marker iNOS in murine macrophages. **B**) FACs analysis showing that AVS100 prevented the generation of M2 ($CD206^+$) but not M1 ($CD80^+$) macrophages from murine BMDM. **C**) qPCR showing that AVS100 preferentially blocks the induction of M2 genes and partially inhibits M1 genes in primary human macrophages polarized towards M2 (IL-4) and M1 (IFNγ + LPS) respectively. **D**) Western-blot analysis showing that AVS100 leads to the acetylation of c-Myc and the reduction of total c-Myc levels, a transcription factor required for M2 polarization.

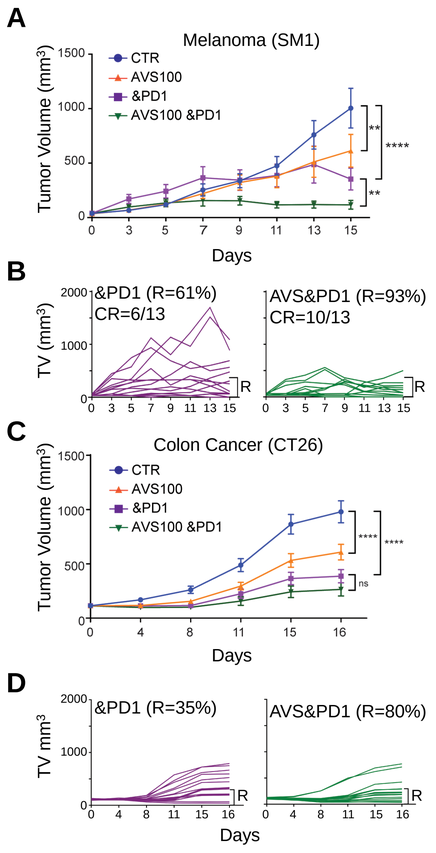

Figure 2. AVS100 has a standalone antitumoral effect and potentiates anti-PD1 therapy

A and C) Effect of AVS100 (100mg/kg) administered daily via oral gavage in combination with anti-PD1 on SM1 melanoma or CT26 colon cancer tumor growth. **B and D**) Analysis of tumor volume in individual mice R= Responders and CR= Complete Responders in which tumor was completely eliminated.
AVS100 had a standalone anti-tumor effect and improved the anti-tumoral efficacy of anti-PD1 therapy

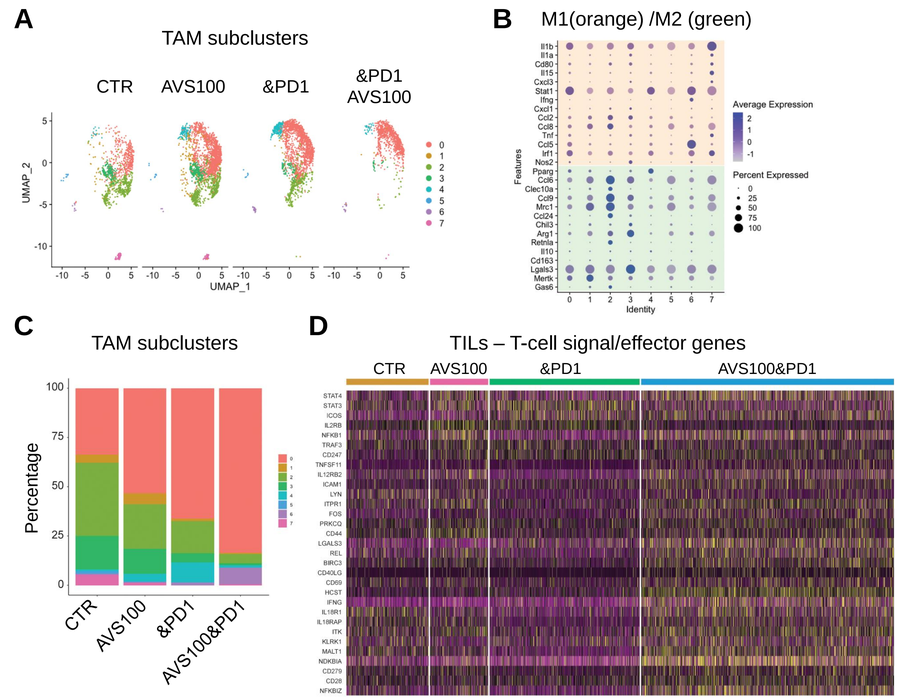

Figure 3. AVS100 reduces anti-inflammatory (M2-like), increases inflammatory (M1-like) TAMs, and increases the T-cell effector signature in Tumor infiltrating lymphocytes

A and C) scRNAseq analysis of Tumor Associated Macrophage (TAM) subsets in SM1 tumors. **B**). M1/M2 gene signature of each TAM subcluster. An additive increase of M1-like subcluster (0) and a reduction of M2-like subclusters (2 and 3) is observed by anti-PD1 and AVS100 treatments. **D**) T-cell effector signature genes in intratumoral T-cells showing elevated expression in combination anti-PD1+AVS100 treatment.

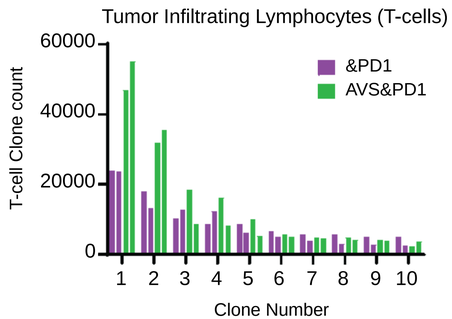

Figure 4. AVS100 expands the immunodominant T-cell clones in TILs

TCRseq analysis of TILs showing the abundance of the 10 most immunodominant T-cell clones. Combination AVS100 &PD1 therapy shows increased expansion of the 2 most immunodominant T-cell clones.