Inhibiting Ehmt2/Ezh2 histone methyltransferases enhances immune microenvironment in a *Trp53*$^{-/-}$ murine ovarian cancer model

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Background

- Ovarian high grade serous carcinoma (HGSC) is a poor prognosis disease with mutations in *TP53* found in around c.100% of all cases[1].
- Ovarian cancer prognosis is strongly associated with development of an anti-tumour immune response[2]. However, tumours can employ epigenetic mechanisms to silence immunostimulatory genes, such as chemokines[3].
- We investigated whether a novel dual inhibitor of the histone methyltransferases Ehmt2/Ezh2 was able to derepress expression of critical chemokines and augment the immune response in a murine ovarian cancer model.

Methods

- *Trp53*$^{-/-}$ ID8 cells were previously generated by CRISPR-Cas9 technique[4].
- An 84-chemokine RT-qPCR array was used to analyse *in vitro* transcript changes following treatment with an Ehmt2/Ezh2 inhibitor.
- C57BL/6 female mice were inoculated with *Trp53*$^{-/-}$ ID8 cells intraperitoneally (IP). Following this, tumours and haemorrhagic ascites develops approximately 40-45 days post-inoculation. Treatments were delivered IP.
- Following tumour dissociation and digestion, cells were stained for flow cytometry and analysed using Cytek Aurora flow cytometer.

Result 1: Chemokine gene upregulation by Ehmt2/Ezh2 inhibition

- *In vitro*, combination Ehmt2/Ezh2 treatment significantly (p<.05) upregulates the expression of lymphocyte-attractant chemokines, such as *CXCL10* (3-fold), *CXCL9* (22-fold) and *CCL5* (14-fold), after stimulation with IFNγ (fig. 1)

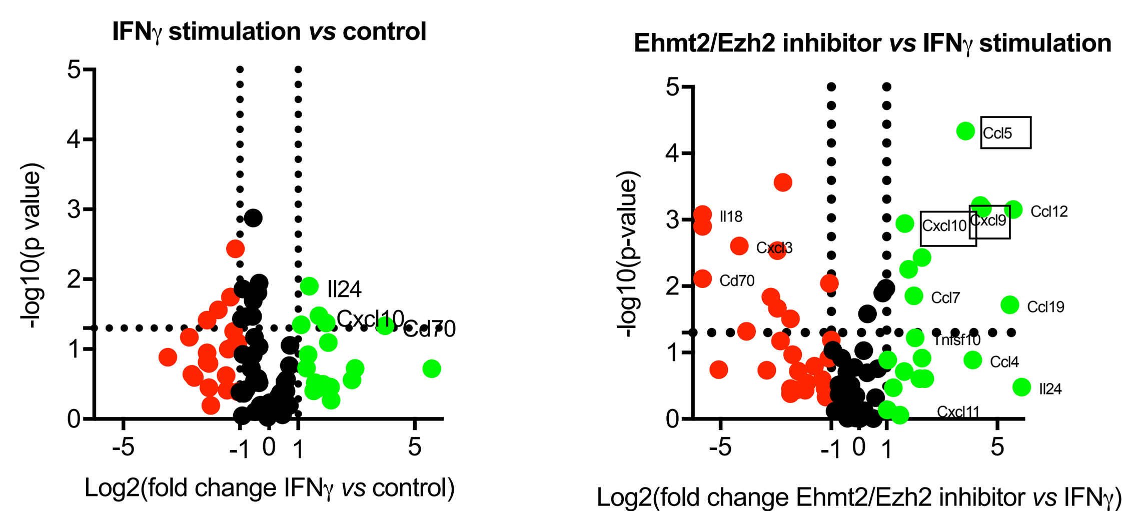

Figure 1: *Trp53*$^{-/-}$ ID8 cells a) stimulated with 1ng/ml IFNγ alone or (b) stimulated with FNγ and treated with 6uM of dual Ehmt2/Ezh2 inhibitor. Green colour denotes increase, red colour decrease and black colour no change.

Result 2: Dual Ehmt2/Ezh2 blockade confers a survival advantage

- *In vivo*, dual Ehmt2/Ezh2 inhibition confers a survival advantage over control (52d *vs* 45d, p<.0001) (fig 2a). Tumour weight and ascites volume were significantly lower in the group treated with the dual inhibitor (mean 120mg vs 178mg, p=.006; 3.7ml vs 5.6ml, p=.003) compared to control (fig 2b).

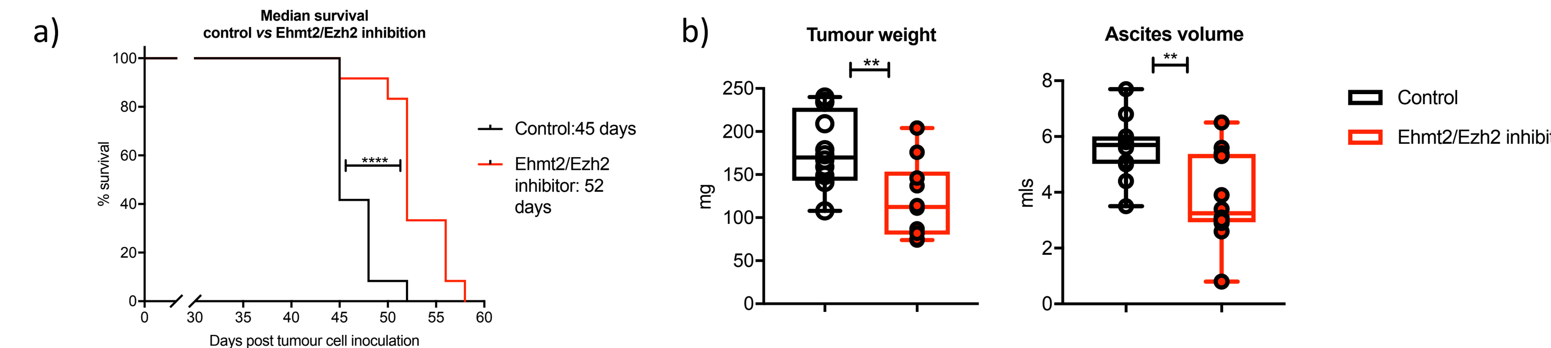

Figure 2: Female C57BL/6 mice were injected IP with *Trp53*$^{-/-}$ ID8 cells on day 0 and treated with a dual Ehmt2/Ezh2 inhibitor 20mg/kg BD (n=12) or vehicle (0.9% NaCl,1% Tween and 3.5% DMSO, n=12) for 14 days via IP injection. Subsequently, mice were allowed to reach humane endpoint. At endpoint, weight and ascites volume were measured.

Result 3: Ehmt2/Ezh2 inhibition shapes the immune microenvironment within tumour deposits

- Ehmt2/Ezh2 inhibition results in an increase in effector CD8^{+} T cells (71.2% vs 54.4% p=.03) with a simultaneous decrease in naïve CD8^{+} T cells (3.44% *vs* 0.68%, p=.02) in the TME (fig 3a). There are significant increases in both mean fluorescence intensity (MFI) of CXCR3, the receptor involved in CXCL9/CXCL10 axis (MFI 3959 vs 1862, p<.001) and granzyme-B producing CD8^{+} cells (65.1% vs 27.2% p<.0001) following Ehmt2/Ezh2 inhibition (fig 3b)

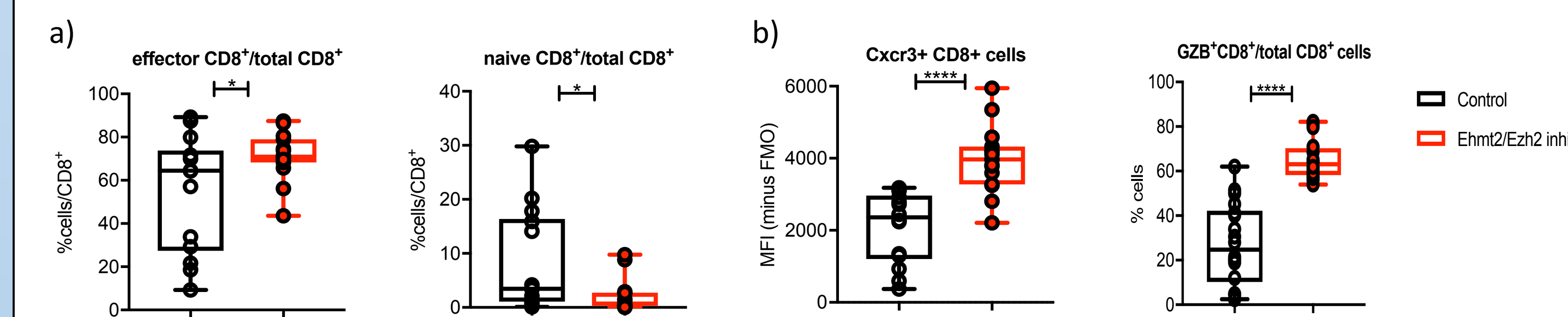

Figure 3: a) Quantitative analysis of flow cytometry performed on mouse tumours, gating on (a) CD8^{+} T cells stained with CD44 and CD62L markers for identification of effector (CD44^{+}CD62^{-}) and naïve (CD44^{-}CD62L^{+}) populations, (b) mean fluorescence intensity of CXCR3 receptor on CD8^{+} cells and CD8^{+}T cells stained with granzyme B antibody following stimulation with PMA/ionomycin, followed by brefeldin A/monensin treatment.

- Treatment with Ezhmt2/Ezh2 inhibitor results in an increase in both Natural Killer (NK) cells (5.2x10^{6} *vs* 2.8x10^{6} cells/g, p=.007) and their CXCR3 receptor expression (MFI 1507 *vs* 440, p<.001) (fig 4).

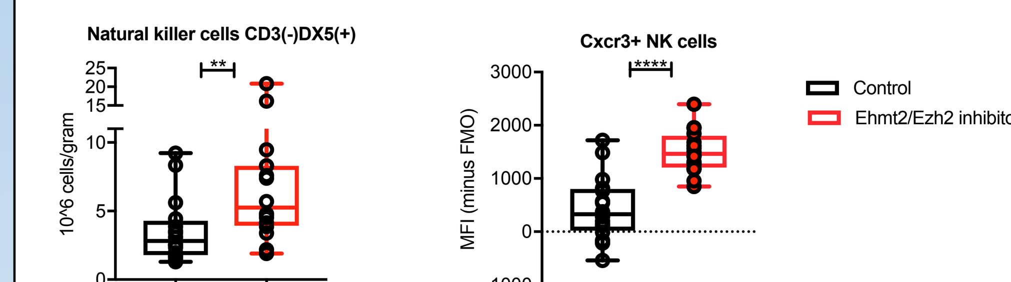

Figure 4: Quantitative analysis of flow cytometry gating on CD3^{-}DX5^{+} for identification of natural killer population (NK cells) and MFI of CXCR3 receptor on NK cells.

- Ehmt2/Ezh2 inhibition reduces the immunosuppressive population of FoxP3^{+}CD4^{+} Treg cells (0.9x10^{6} vs 2.2x110^{6} cells/g, p=.02).

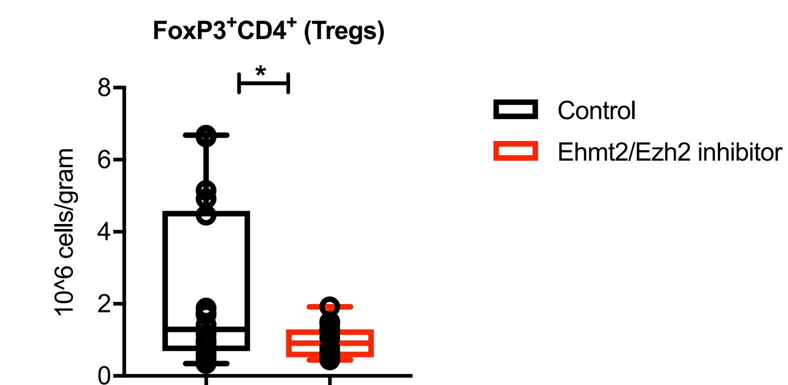

Figure 5: Quantitative analysis of flow cytometry gating on CD4^{+} T cells stained with FoxP3 marker.

- Treatment with Ehmt2/Ezh2 inhibitor increases the number of dendritic cells (DCs) (3.3x10^{6} *vs* 1.3x10^{6} cells/mg, p=.0003). These DCs have higher expression levels of the activating receptor CD86, compared to control (mean fluorescence intensity 22365 *vs* 16181, p=0.009) (fig 6).

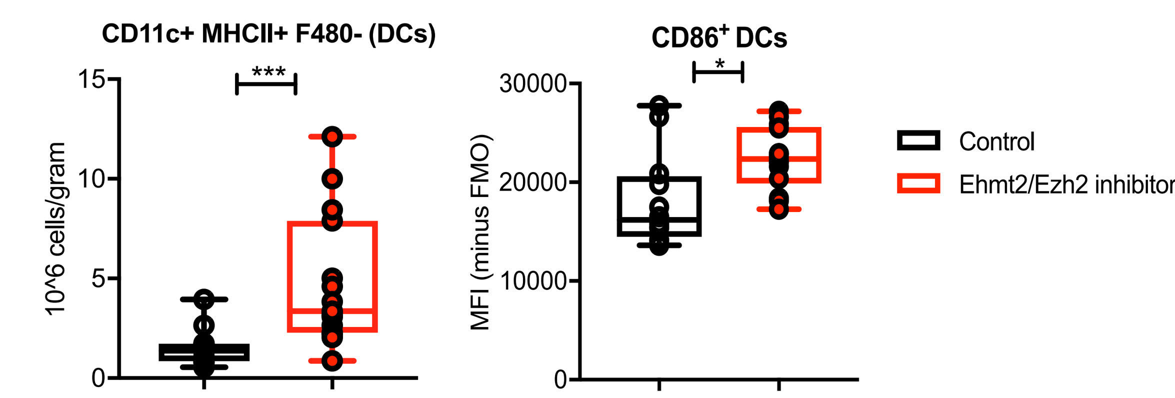

Figure 6: Quantitative analysis of flow cytometry gating on CD11b^{+}Ly6G^{-}SiglecF^{-}F4/80^{-}MHCII^{+}CD11c^{+} cells (DCs) and quantification of CD86 MFI activation marker on DCs.

Result 4: Ehmt2/Ezh2 inhibition augments activation of splenic CD8^{+} cells

- CD8^{+} cells derived from mice after treatment with Ehmt2/Ezh2 inhibitor demonstrate higher level of perforin (10.2% vs 5.9%, p=.004) and IFNγ (22% vs 18%, p =.01), compared to control.

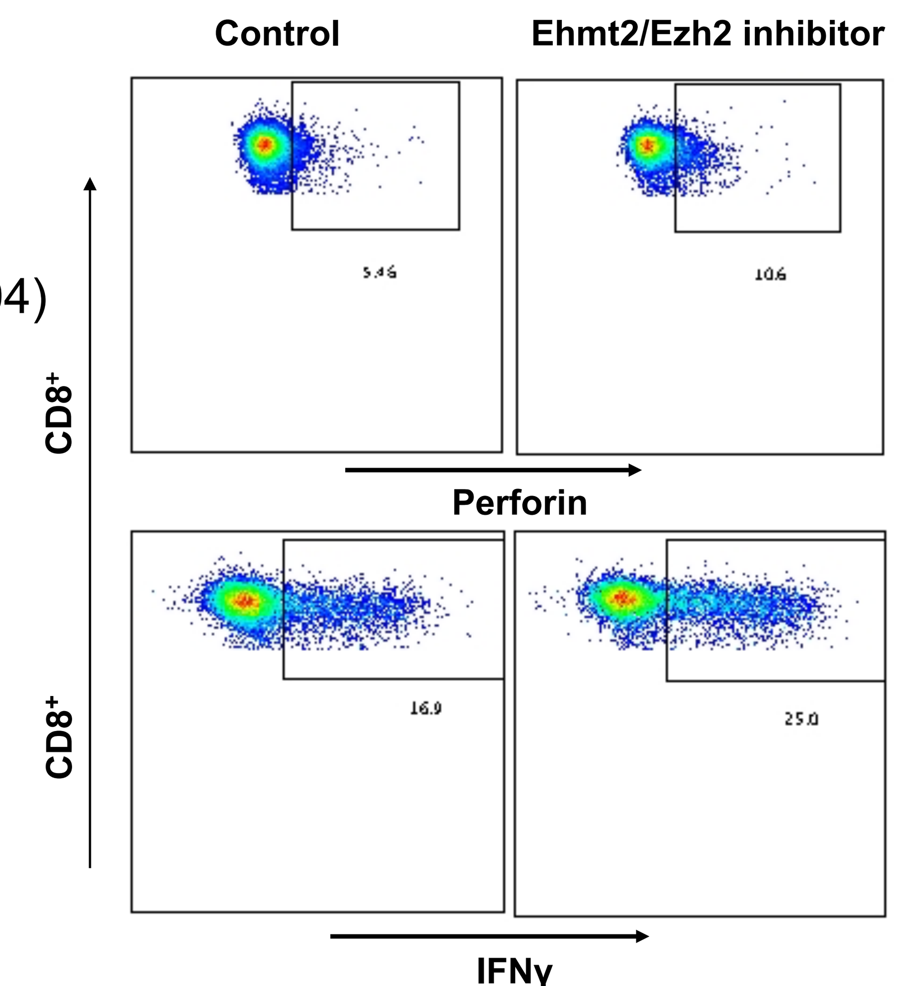

Figure 7: Mouse spleenocytes were stimulated with PMA/ionomycin, treated with brefeldin A/monensin and then subjected to flow analysis after stained with intracellular markers against Perforin and IFNγ. Plots are showing a representative sample closest to the mean and numbers represent frequencies respective to entire CD8^{+} population.

Conclusion

Inhibition of Ehmt2/Ezh2 stimulates expression of chemokines involved in T cell, NK and DC recruitment. *In vivo*, Ehmt2/Ezh2 inhibition alters the immune microenvironment and confers a survival benefit. This suggests that Ehmt2/Ezh2 inhibition could augment the anti-tumour immune response in ovarian cancer.

[1]Ahmed et al J Pathol 2010; [2]Ovarian Tumor Tissue Analysis C. JAMA Oncol. 2017; [3]Chiappinelli KB et al. Cancer Res. 2016; [4]Walton J et al. Cancer Res. 2016.

Disclosures: All authors have nothing to declare

Poster presentation number: 1946P