Combination of Ruxolitinib with Resminostat exerts antitumor effects in a chicken embryo metastasis model

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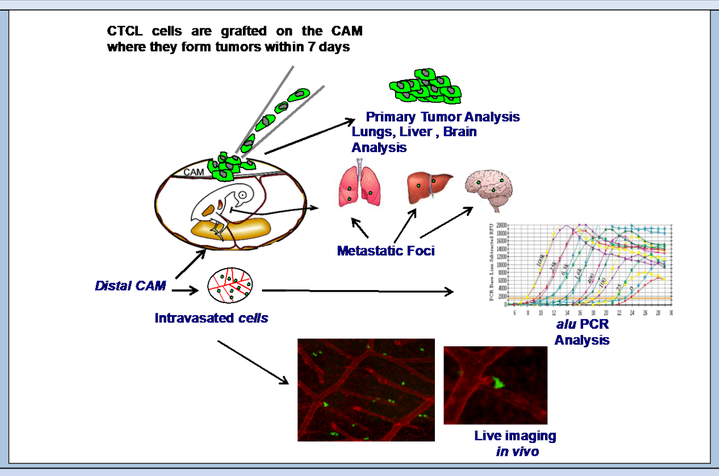
INTRODUCTION & OBJECTIVES

Elucidation of molecular targets will improve the clinical management of cutaneous T-cell lymphomas (CTCL). The combination of JAK/HDAC inhibitors has exerted beneficial effects in haematological malignancies, presenting promising therapeutic CTCL targets. Our current published data (1) showed that the combination of **Resminostat** (HDACi) with **Ruxolitinib** (JAKi) had cytotoxic effect, inhibited proliferation in CTCL cell lines suggesting a strong synergy for both drugs. The drugs' combination inhibited phosphorylation of STAT3, Akt, ERK1/2 and JNK in MyLa, while it reduced activation of Akt and JNK in SeAx. It is challenging to explore the effect of JAK/HDACi in tumor formation, angiogenesis and metastasis in CTCL.

AIMS: We used **a CTCL *in vivo* chicken embryo model** (2), in order to study the **effect of Resminostat and/or Ruxolitinib** in the **possibility of generating primary tumors and studying its metastatic potential** in a timely and cost-effective manner offering a number of unique advantages to study the multistep process of tumor cell metastasis. Thus, we generated xenografted tumors derived from MyLa and SeAx cells implanted on top of the chicken chorioallantoic membrane (CAM). This new CTCL pre-clinical model is a suitable *in vivo* model to test new combination of antitumor agents to improve CTCL patient treatment

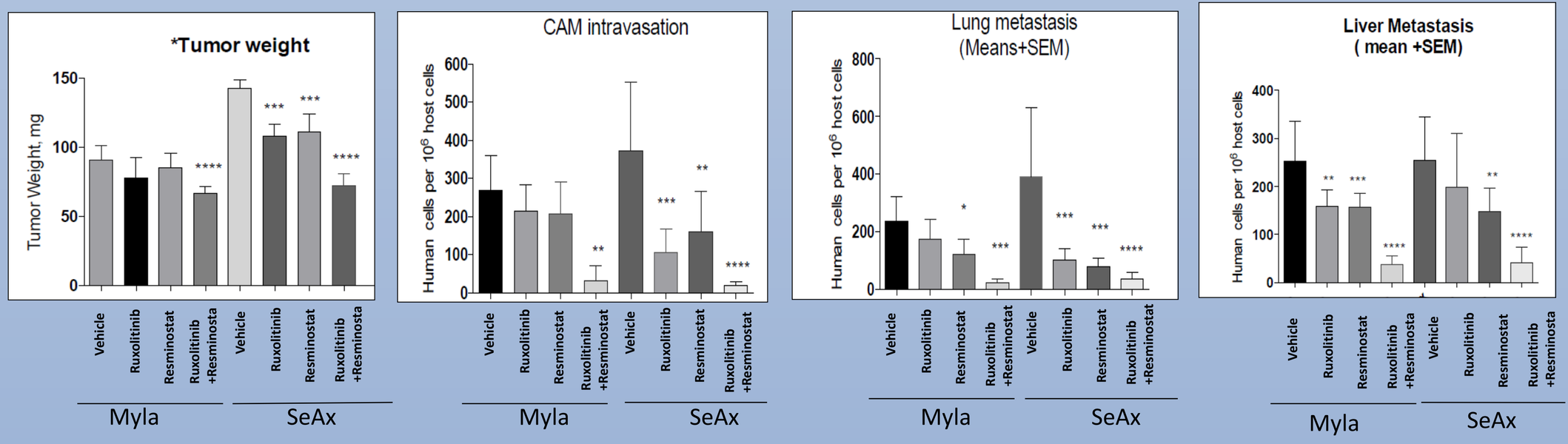
MATERIALS AND METHODS

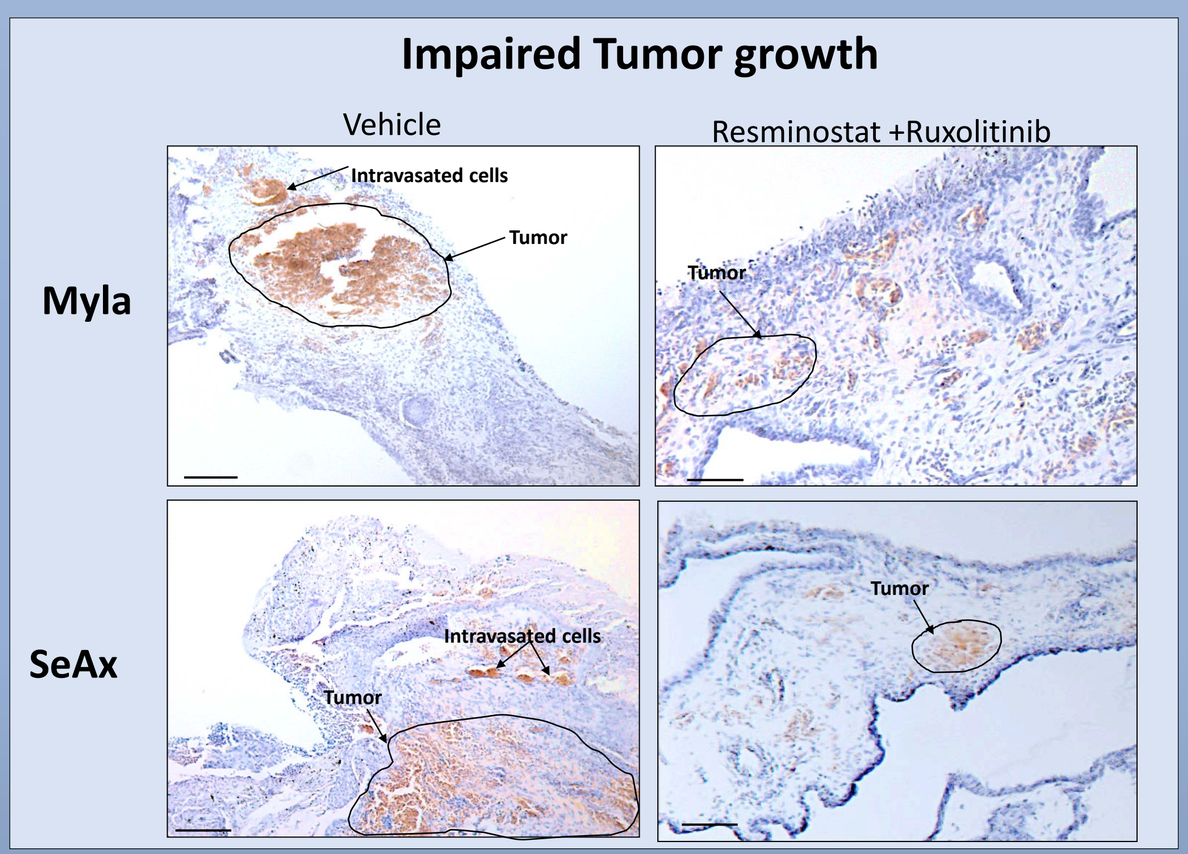
To study the CTCL tumorigenesis and metastasis, 10^6 Myla or SeAx cells were injected on CAM embryos. Tumors had been grown very well and spontaneous metastasis in chick embryos was performed. To study the impact of JAKi and HDACi in Myla and SeAx cells, we used topically 15μM and 5μM respectively every two days. We harvested on day 7 and analyzed the number of tumor cells on the CAM, liver and lung by specific Alu PCR. Primary tumors were excised and weighed. The portions of the CAM, liver and lung were harvested and analyzed for the number of human cells by qAlu PCR. Immunohistological analysis of CTCL tumors were performed using CD44 antibody (brown staining) and tissue was counterstained with hematoxylin. For the inmunofluorescence analysis *in vivo* CTCL cells were pre-labeled with green fluorescence CellTracker, CAM vasculature was highlighted by injecting Rodhamine (vessels:red).

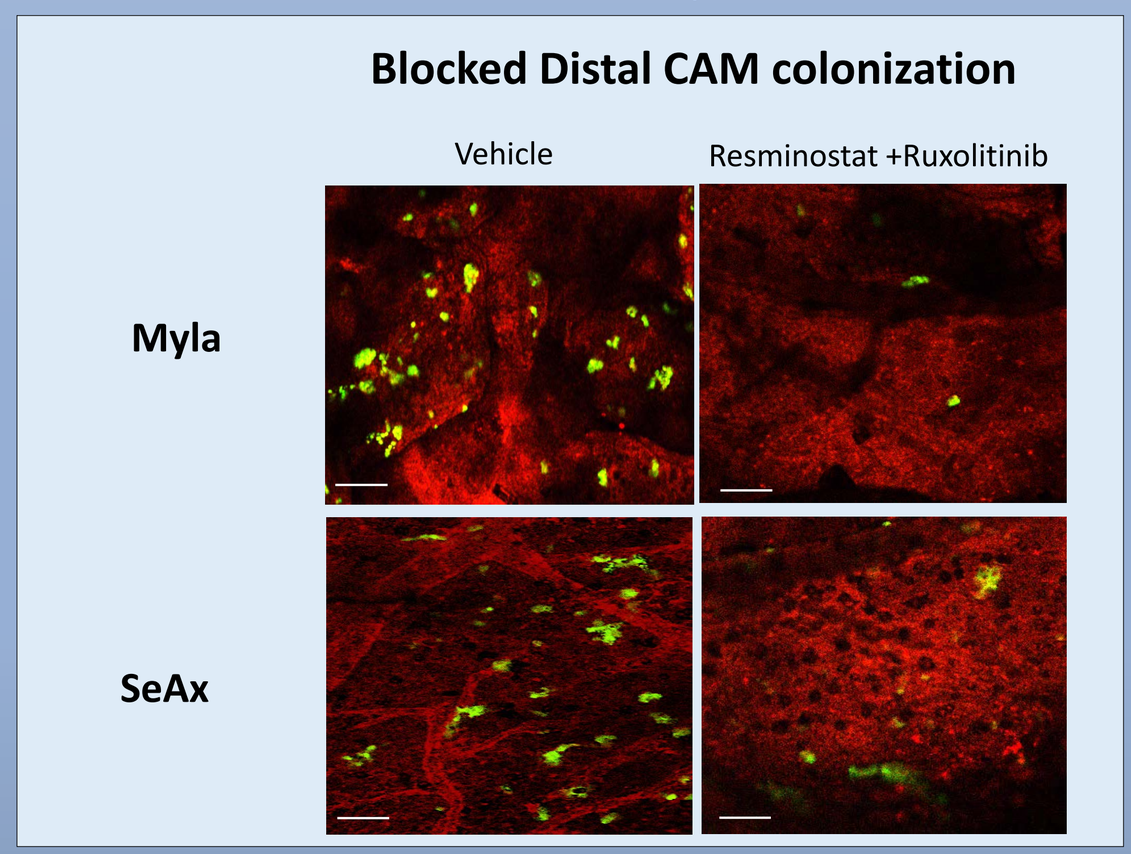


RESULTS

In vivo, JAKi and HDACi monotheraphy inhibited primary tumor formation and CAM intravasation in CTCL cell lines but remarkably **the combination of resminostat with ruxolitinib was more effective in inhibiting the primary tumor formation** and **blocked CAM intravasation** as well as **liver and lung metastasis**. Combination of JAKi/HDACi greatly impaired primary tumor growth. Moreover, we detected that control MyLa and SeAx cells intravasated the distal CAM and disseminated to internal organs, lung and liver, forming secondary metastatic foci**. Combination of resminostat/ruxolitinib blocked CAM intravasation as well as liver and lung metastasis**. Data show mean SD of three independent experiments. * $p < 0.05$, ** $p < 0.01$,*** $p < 0.005$, **** $p < 0.001$ by two- tailed unpaired Student-test.







CONCLUSION

Our results using the chick embryo CTCL spontaneous metastasis model indicated that **the JAKi/HDACi combination exhibited synergistic antitumoral effects and blocked CAM intravasation, as well as liver and lung metastasis**, and may represent **a promising novel therapeutic modality for CTCL patients**. Importantly, we highlight that *in vivo* chick embryo metastasis model could be **a good CTCL pre-clinical model** to discover new treatments to improve CTCL patients survival.

REFERENCES

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