Salicylates Potentiate and Broaden CRM1 Inhibitor Anti-Tumor Activity via S-Phase Arrest and Impaired DNA-Damage Repair

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Introduction

- Chromosome region maintenance protein1 (CRM1) mediates protein export from the nucleus and is a new target for anti-cancer therapeutics.
- Broader application of KPT-330 (selinexor), a first-in-class CRM1 inhibitor recently approved for multiple myeloma and diffuse large B-cell lymphoma (DLBCL), has been limited by substantial adverse effects (AEs).
- To address this clinical problem, we focused on identifying novel strategies to boost the potency, reduce toxicity, and broaden the applicability of CRM1 inhibitors to a wider range of malignancies.

Results

- We discovered that salicylates could markedly enhance the anti-tumor activity of CRM1 inhibitors by extending the mechanisms of action beyond CRM1 inhibition.
- KPT-330 was chosen as the prototypical CRM1 inhibitor given its current FDA approval status and characterized pharmacokinetics; and choline salicylate (CS) was chosen as the prototypical salicylate given its favorable pharmacokinetics and reduced antiplatelet, renal, neurological and gastrointestinal AEs in humans compared to other salicylates.
- By using cell lines belonging to different hematologic malignancies and solid tumors, we demonstrated *ex vivo* that the combination of KPT-330 and CS (K+CS) could induce unique and significant antitumor effect at much lower dose of KPT-330 (at 25% of the dose used in the clinic), thereby potentially mitigating prohibitive clinical AEs **(Figure 1A-E)**.
- This significant synergetic antitumor effect observed with K+CS *ex vivo* was also validated *in vivo* by using an NSG mouse model of mantle cell lymphoma **(Figure 1F and G)**.

Results

- The K+CS combination did not show this potent toxic effect on non-malignant cells *in vivo* and was safe without inducing toxicity to normal organs in NSG mice.

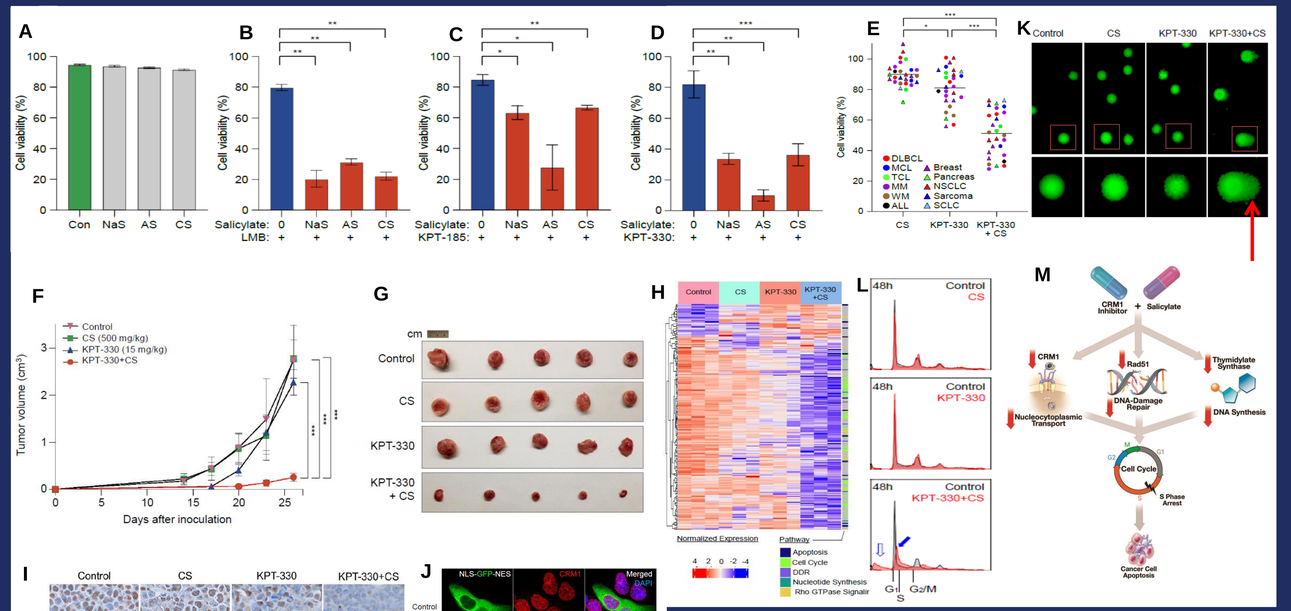
Mechanisms

- Protein profiling through mass spectroscopy revealed that K+CS uniquely affects the cellular pathways of DNA-damage repair, DNA synthesis and cell cycle progression **(Figure 1H).**
- Studies involving immunoblotting, cell cycle analysis, immunofluorescence microscopy assessing nucleocytoplasmic molecular export and DNA damage, reporter assay to assess homologous recombination repair proficiency and immunohistochemistry showed that, compared to KPT-330 treatment alone, K+CS decreased the expression of CRM1, Rad51 and thymidylate synthase proteins *in vitro* and *in vivo* **(Figure 1I)**, leading to more efficient inhibition of CRM1-mediated nuclear export **(Figure 1J)**, impairment of DNA-damage repair **(Figure 1K, comet assay showing DNA damage which is represented by the comet tail-red arrow)**, reduced pyrimidine synthesis, and importantly a unique cell cycle arrest in S-phase, thus leading to cell apoptosis **[Figure 1L; progression of S-phase arrest with time was observed upon KPT-330+CS (blue filled arrow) while increasing cell death (blue hollow arrow)]** .
- Pathway analyses through RNA sequencing also paralleled the findings of proteomic studies.

Primary patient samples

- Importantly, K+CS treatment exerted unique and significant antitumor effect *ex vivo* on primary malignant cells obtained from patient's with high-risk hematologic malignancies such as double hit DLBCL, BTK and BCL2 inhibitor resistant mantle cell lymphoma, TP53 deleted/mutated chronic lymphocytic leukemia and high-risk multiple myeloma, and also on PDX models of ovarian cancer and glioma.

Figure 1



Conclusions

- K+CS is a unique all-oral drug combination with a novel constellation of mechanisms of action on cellular pathways that are exploited by cancer cells **(Figure 1M, visual summary)**.
- K+CS represents a new class of therapy for multiple cancer types and will stimulate future investigations to exploit DNA-damage repair and nucleocytoplasmic transport for therapy of blood cancers.