The glycogen synthase kinase-3β inhibitor 9-ING-41 in combination with chemoimmunotherapy provides long-term survival in the Th-MYCN mouse model

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INTRODUCTION

Neuroblastoma is the most common extracranial solid tumor in children. Survival rates of children with high-risk disease are ~50%, reducing to 10% for relapsed/refractory disease, highlighting the need for more effective treatments. Glycogen synthase kinase-3β (GSK-3β) is a serine/threonine kinase, highly expressed in cancers including neuroblastoma. In addition to its well-established role in driving cell growth and proliferation, there is increasing evidence suggesting a role in supporting tumor immune evasion. 9-ING-41 (Elraglusib), a GSK-3β inhibitor with clinical activity in adult cancers, is effective as a single agent in neuroblastoma animal models. However, the efficacy of 9-ING-41 in combination with current clinically relevant chemotherapy and chemoimmunotherapy neuroblastoma protocols that include anti-GD2 antibody for high-risk patients, has not been established.

AIMS

To establish, in the Th-MYCN mouse model of neuroblastoma, clinically relevant chemoimmunotherapy treatment protocols that include anti-GD2 antibody (14G2a), and to evaluate whether and how 9-ING-41 enhances their efficacy.

METHODS

Th-MYCN transgenic mouse and Kelly human xenograft models of neuroblastoma were used to develop clinically relevant chemoimmunotherapy regimens and to evaluate the efficacy of 9-ING-41. Pharmacokinetic studies with radiolabelled anti-GD2 antibody 14G2a were performed to ensure that dosing mirrored dinutuximab serum levels achieved clinically. To provide mechanistic insights, efficacy was compared between Th-MYCN allografts in immunocompetent 129/SvJ and immunodeficient Balb/c nude and NSG mice to determine immune dependence, and changes in the tumor immune microenvironment were evaluated through multi-parameter flow cytometry.

RESULTS

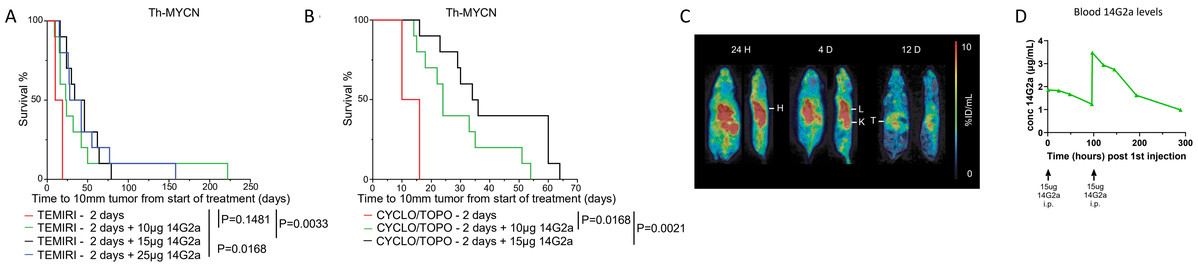

Figure 1. Modelling current standard-of-care chemoimmunotherapy for neuroblastoma
Th-MYCN mice relapsed within 75 days following treatment with 2 consecutive daily doses of temozolomide/irinotecan (TEMIRI) (A) or cyclophosphamide/topotecan (CYCLO/TOPO) (B), in addition to 15 µg 14G2a on day 1 and day 5. Pharmacokinetics of radiolabelled 14G2a confirmed that this regimen resulted in equivalent 14G2a serum levels in tumor-bearing TH-MYCN mice as observed for Dinutuximab in neuroblastoma patients (C and D).

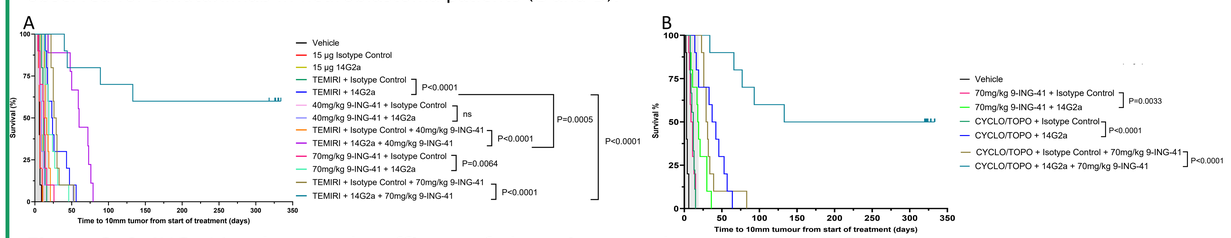

Figure 2. 9-ING-41 enhances the efficacy of chemoimmunotherapy in Th-MYCN mice
Addition of 9-ING-41 to TEMIRI or combined TEMIRI/14G2a, delayed tumor growth in a dose-dependent manner, resulting in significantly extended survival compared to mice receiving the same treatments without 9-ING-41 (A). 6/10 mice treated with TEMIRI/14G2a/70 mg/kg 9-ING-41 were long-term tumor-free survivors at 1 year of age (P<0.0001). These results were replicated on a CYCLO/TOPO backbone (B).

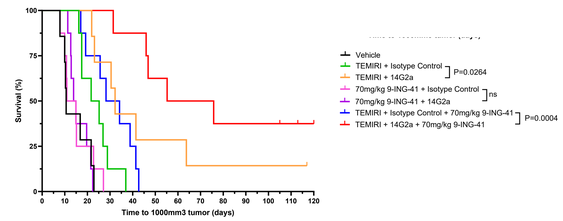

Figure 3. 9-ING-41 enhances the efficacy of chemoimmunotherapy in a human neuroblastoma xenograft model
In Kelly cell line xenografts in Balb/c nude mice, addition of 14G2a to TEMIRI significantly increased survival compared to TEMIRI/isotype control from 24 to 32 days. However, 9-ING-41 alone did not significantly extend survival compared to vehicle treatment (P=0.6966), nor did 9-ING-41 enhance the efficacy of 14G2a (P=0.8312). In contrast, the combination of 9-ING-41 with TEMIRI is able to boost anti-GD2 mAb efficacy (P=0.0004).

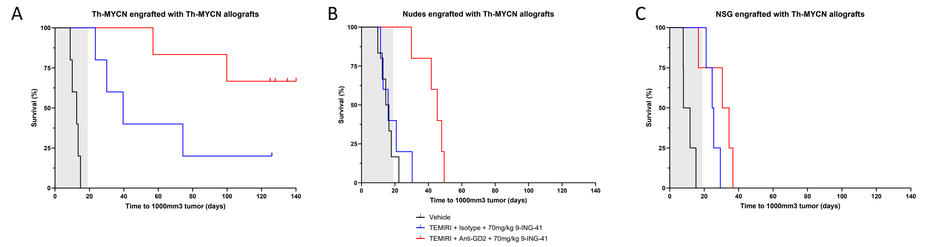

Figure 4. Optimal activity of TEMIRI/9-ING-41 requires an intact immune system
Th-MYCN tumor cells allografted into immunocompetent 129/SvJ littermates (A) and treated with TEMIRI/14G2a/9-ING-41 significantly extended survival with 4/6 mice not relapsing with a tumor. Survival was also significantly extended in Balb/c nude mice (lacking T-cells) (B), but not in NSG mice (deficient in T-, B- and NK-cells) (C) highlighting the importance of an intact immune system in anti-GD2 therapy.

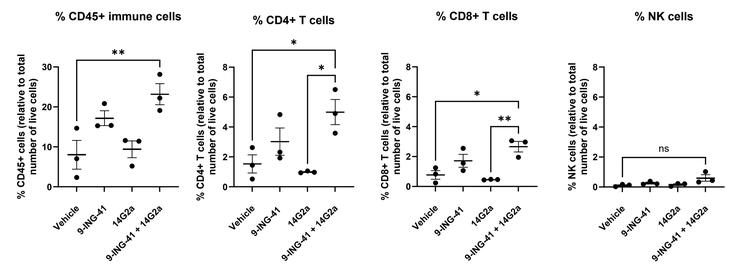

Figure 5. Combined 9-ING-41/anti-GD2 monoclonal antibody treatment significantly increases CD4+ and CD8+ T-cell proportions in Th-MYCN tumors
Using multi-parameter flow cytometry, trends were observed for 9-ING-41 alone increasing CD4+ and CD8+ T cell proportions within treated tumors. Only upon combining 9-ING-41 with 14G2a were CD4+ and CD8+ T cell proportions significantly increased. A trend for increased NK cell levels was also found after 9-ING-41/14G2a treatment.

CONCLUSIONS

This study provides compelling preclinical evidence for incorporating GSK-3β inhibition into established treatment backbones and supports clinical evaluation of 9-ING-41 combination therapy to improve outcomes for children with high-risk neuroblastoma.

ACKNOWLEDGEMENTS

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