9-ING-41, a selective small molecule inhibitor of Glycogen Synthase Kinase-3 beta (GSK-3beta), may enhance neuroblastoma immunogenicity

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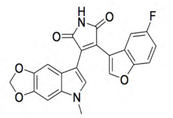
Introduction

- **Neuroblastoma (NBL)**: an embryonic tumor originating from neural crest precursor cells
  - one of the **most prevalent pediatric solid tumors**, estimated five-year progression free survival < 50% in stage-4 patients
- NBL is **refractory to T-cell mediated therapies** (e.g., checkpoint inhibition)
  - Poorly immunogenic tumor: **extremely low peptide/MHC-I complex expression** of NBL cells[1]
  - NBL might be sensitive to combination therapy of checkpoint inhibition with a selective small molecule inhibitor
- **GSK-3β** overexpressed by NBL: therapeutic target
  - may regulate peptide/MHC-I complex display on tumor cell surface[2]
- **9-ING-41: selective small molecule inhibitor of GSK-3 β** (figure 1)
  - Broad spectrum pre-clinical anti-cancer activity (incl. NBL)[3,4,5]
  - nd safety profile in adult patients with es in children underway)



3-(5-Fluoro-benzofuran-3-yl)-4-(5-methyl-5H-[1,3]dioxolo[4,5-f]indol-7- yl)-pyrrole-2,5-dione

Figure 1: Chemical structure representation of 9-ING-41

Methods

- NBL cell lines cultured for 24 or 72 hours with IFN-γ and/or 9-ING-41
  - **GIMEN, SH-SY5Y → MYCN-non amplified**
  - **SK-N-BE, and IMR32 NBL line → MYCN amplified**
- Measure cell surface expression of MHC-I and PD-L1 using multicolor flow cytometry analyses

Results

- 9-ING-41 **increased IFN-γ-mediated MHC-I surface expression on all NBL cell lines** tested, specifically after 72 hours of incubation (figure 2)
- Most pronounced effects on cell lines without MYCN amplification (figure 2A)
- PD-L1 expression was also upregulated in two of the cell lines (figure 2A) → **9-ING-41 combined with PD-1/PD-L1 immune checkpoint blockade is worthy of further investigation**

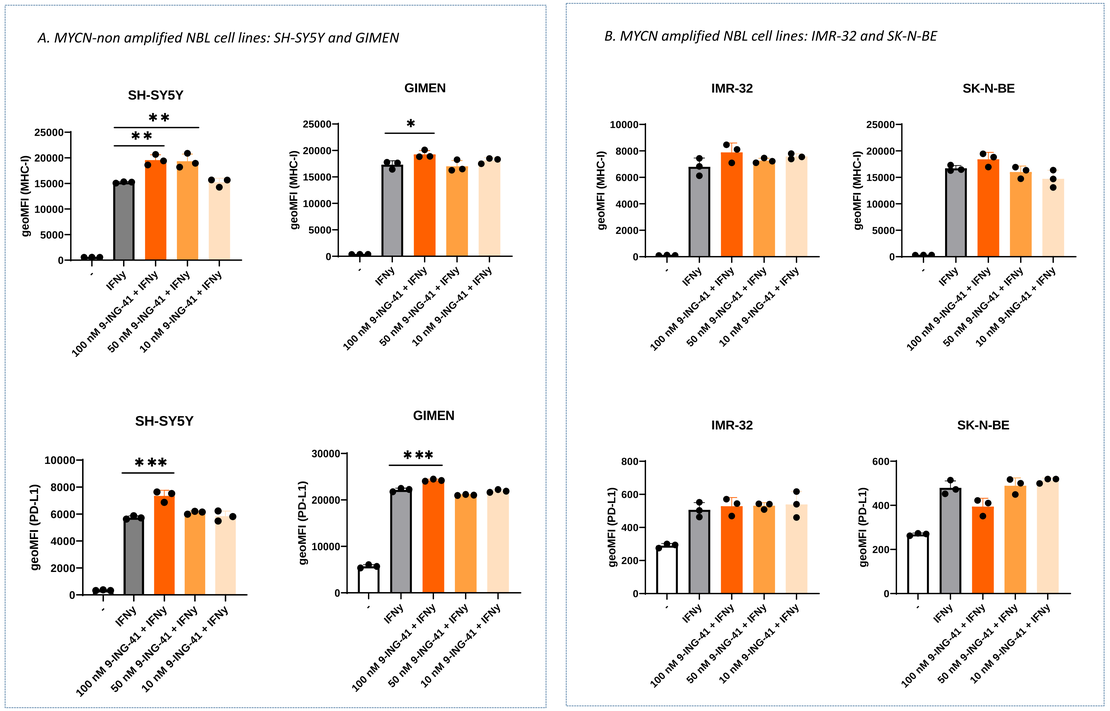


*Figure 2: Flow cytometry data of non NMYC amplified (SH-SY5Y and GIMEN) (A) and NMYC amplified NBL cell lines (IMR-32 and SK-N-BE) (B) after 72h co-treatment with IFNγ (25ng/ml). *p<0.05, **p<0.01, ***p<0.001 compared to IFNγ condition using one-way ANOVA followed by Dunnett's multiple comparison. Data shown as mean ± SD*

Conclusion

- **9-ING-41 may facilitate recognition and killing of NBL by CTL and support the further development of 9-ING-41 as a potential treatment for NBL** (figure 3)

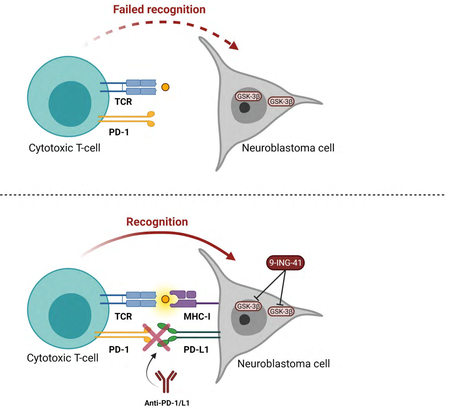


Figure 3: Cytotoxic T-cells (CTLs) fail to recognize neuroblastoma (NBL) cells, as these have extremely low MHC-I expression. Inhibiting GSK-3ß activity with 9-ING-41 increases MHC-1 expression and, in some cases, PD-L1 expression on NBL cells. 9-ING-41 combined with anti-PD-1/L1 therapy can potentially increase recognition and killing by CTLs.

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