A NOVEL PRO-APOPTOTIC-BASED COMBINATION THERAPY FOR PEDIATRIC SOLID TUMOR TREATMENT

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INTRODUCTION

Despite advances in pediatric oncology, solid tumors - accounting for ~30% of childhood cancers - remain a leading cause of mortality due, in part, to late diagnosis, therapeutic resistance, and a lack of effective treatment options [1,2]. Current disease management remains suboptimal, which underscores the need for innovative therapeutic strategies to overcome associated challenges. One promising approach involves nanomedicine, which offers enhanced drug delivery and efficacy. For example, a Phase II clinical trial revealed that ~30% of Ewing's sarcoma patients responded favorably to the chemotherapeutic nanomedicine Abraxane® [NCT02945800]; however, elevated levels of anti-apoptotic proteins (targetable by BCL-2 inhibitors) associated with treatment resistance. Although BCL-2 inhibitors have progressed to Phase I and II clinical trials, their clinical use has been limited by dose-limiting toxicities (thrombocytopenia in particular) [3]. To circumvent this problem, we evaluated polymer therapeutics as a modular, on-demand platform for combination therapy development in pediatric solid tumors [4]; specifically, we evaluated the potential development of polypeptide-based nanoconjugates.

AIMS

- ✓ Identify optimal anti-tumor drug combinations involving BCL-2 inhibitors in two primary pediatric tumor cell lines
- ✓ Employ selected combinations to develop novel nanotherapeutics [5] that support targeted and controlled delivery in pediatric solid tumors

METHODS & RESULTS

1. Material and Methods

We subjected the A673 and RH30 sarcoma-derived cell lines to high throughput screening (HTS) of combinations of BCL-2 inhibitor (Bi) with the Prestwick library (PW; comprising 1120 FDA-approved drugs). We treated cells for 72 h with 2.5 μM PW library compounds alone or in combination with Bi at the IC_{25} value (specific for each cell line) identified in previous studies (data not shown). We automatically added PW compounds using the LiHa Freedom Evo® (Tecan) and evaluated cytotoxicity using the MTS assays. Finally, we calculated synergy scores using the Bliss synergy model (**Figure 1**).

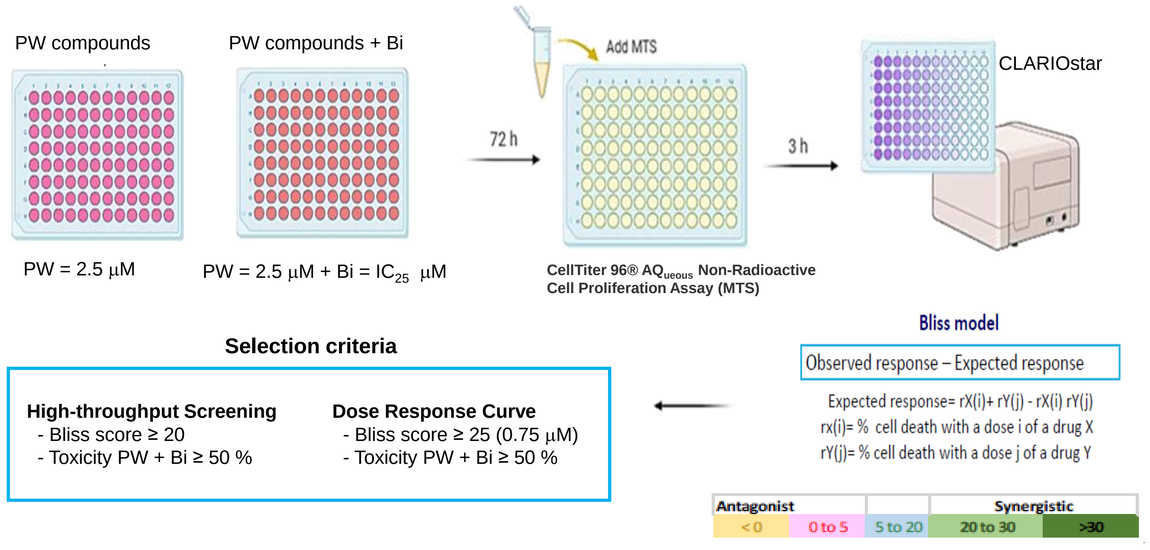
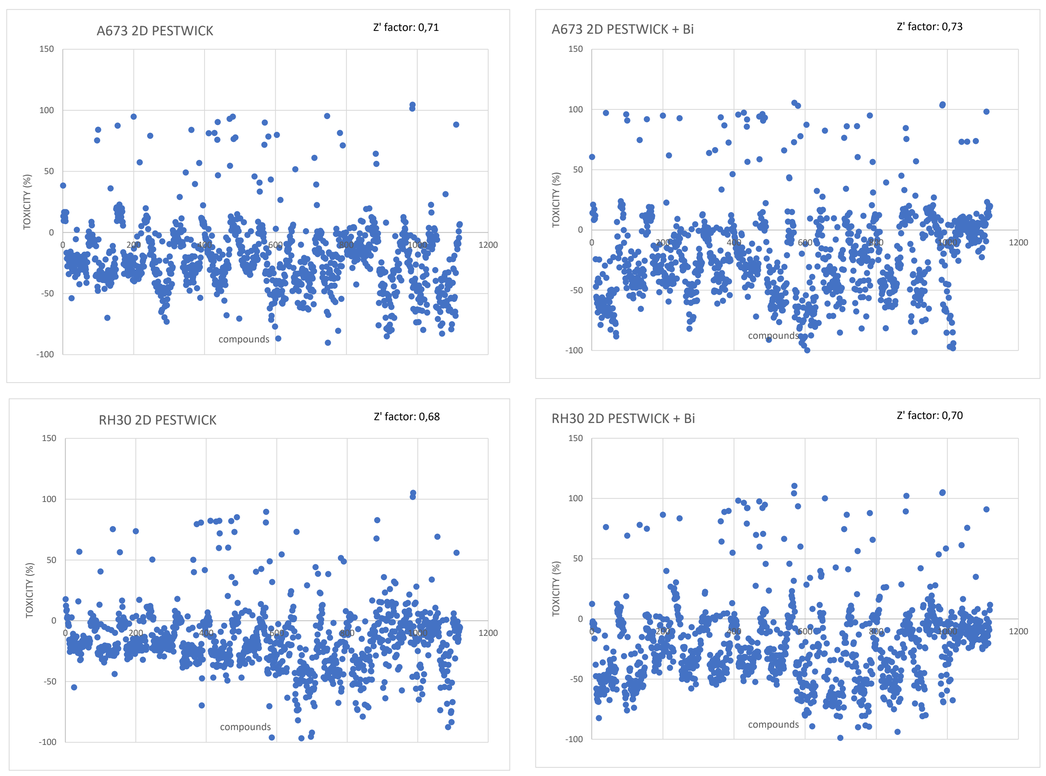


Figure 1. Schematic representation of the methodology and the selection criteria applied.

2. High-throughput drug combination screening with BCL-2 inhibitor

Figure 2A displays the representative results from library compounds in the presence/absence of Bi in both cell lines (A673; upper and Rh30; lower). HTS rendered a list of 16 potential hits (6 for A673; 10 for RH30) that synergized with Bi according to established criteria (**Figure 2B**). We performed dose-response curves to confirm the synergy in both cell lines (**Figure 2C;** Hit 6, rest of data not shown).

A)

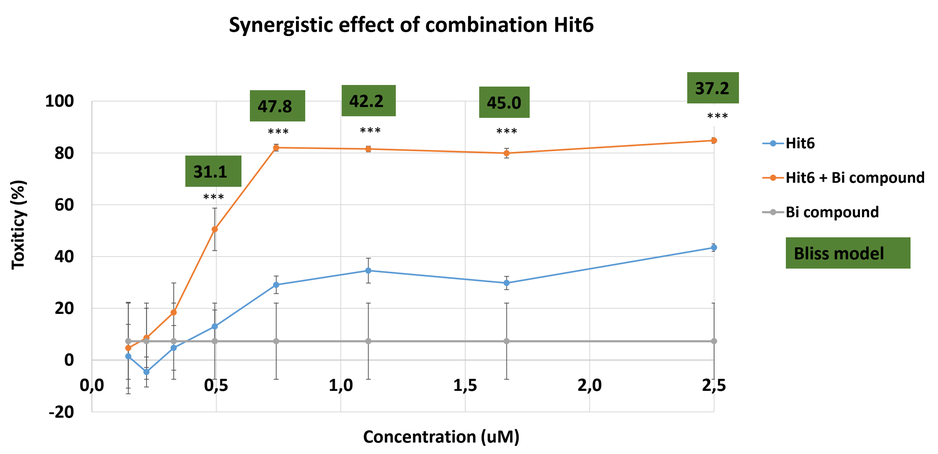


B)

PW Compound	A673 cell line Toxicity (%) PW (2.5 μM)	A673 cell line Toxicity (%) PW+Bi	A673 cell line BLISS-MODEL	RH30 cell line Toxicity (%) PW (2.5 μM)	RH30 cell line Toxicity (%) PW+Bi	RH30 cell line BLISS-MODEL
Hit 1	49	80	26.6	53	89	29.7
Hit 2	35	72	30.8	49	89	32.8
Hit 3	41	75	28.4	59	88	23.8
Hit 4	39	64	20.0	40	87	38.8
Hit 5	13	56	35.2	44	79	34.7
Hit 6	59	85	21.3	62	89	23.5
				46	89	37.0
				65	89	20.5
				47	78	26.6
				54	90	30.9

Antagonist / Synergistic: < 0 | 0 to 5 | 5 to 20 | 20 to 30 | >30

C)



3. Designing polymer-based combination nanoconjugate

Based on dose-response synergy scores (≥25 at 0.75 μM) and the presence of functional groups amenable to conjugation, we selected three lead candidates (two for A673 and one for RH30) for nanoconjugate development (**Figure 3**).

A)

PW Compound	Toxicity (%) PW (0.75 μM)	Toxicity (%) PW+Bi	BLISS-MODEL
Hit 1	34	86	40.4
Hit 6	29	82	47.8
Hit 14	59	87	26.0

B)

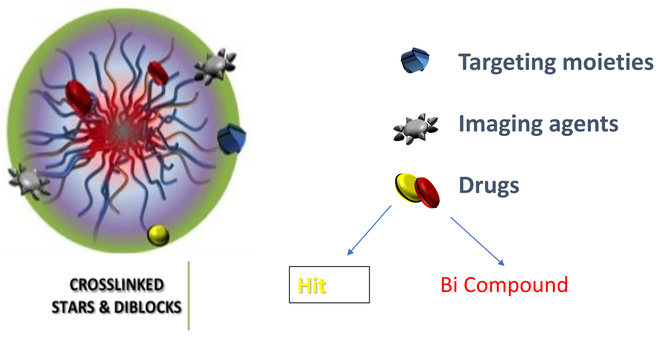


Figure 3. A) Table of selected candidates for conjugation. **B**) Schematic representation of a polymer-based combination nanoconjugate with selected drug combinations.

Figure 2. High-throughput drug combination screening. **A**) Graphical results of combination screening experiment for A673 (upper) and RH30 (lower) cells. *Left panels*: PW monotherapy; *Right panels*: combination therapy PW + Bi. **B**) Toxicity and respective bliss model synergy score of the selected candidates. **C**) Example of the Dose response curve: Hit6 candidate.

CONCLUSIONS & OUTLOOK

We present a list of FDA-approved drug candidates that enhance the anti-tumor effects of BCL-2 inhibition in pediatric solid tumor models; our findings contribute to a foundational dataset of potential therapeutic agents for further preclinical evaluation and provide a rationale for future development of combination nanotherapeutics. Ongoing efforts will focus on engineering nanoconjugates at optimized drug ratios that exhibit maximal synergy in vitro and, ultimately, in vivo.

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