1142-A

A NOVEL ALK-BASED COMBINATION THERAPY FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME

Masiá E.[1,2,3], Martínez-Martínez E.[1] and Vicent M.J.[1,2,3]

[1]Polymer Therapeutics Laboratory, Centro de Investigación Príncipe Felipe (CIPF), Valencia (Spain)
[2]Screening Platform, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain
[3]Centro de Investigación Biomédica en Red Cáncer (CIBERONC)
emasia@cipf.es

INTRODUCTION

Glioblastoma multiforme (GBM) is the most frequent class of malignant primary brain tumor and one of the most aggressive forms of cancer, with affected individuals presenting with a poor prognosis and high rate of relapse[1,2]; therefore, developing new drugs or exploring novel drug combinations is urgently needed. In this context, anaplastic lymphoma kinase (ALK) inhibitors have been postulated as promising candidates for GBM treatment in preclinical studies[3]. As ALK signaling might play an anti-apoptotic role in glioma cells[4] and promote resistance to cancer therapies, combining ALK inhibitors with other cytotoxic drugs could improve treatment efficacy.

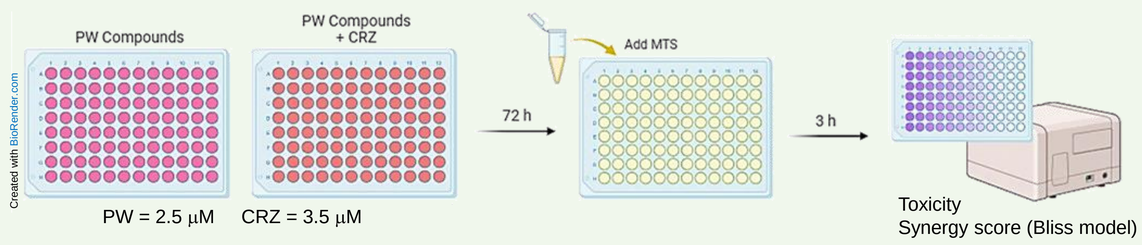
AIMS

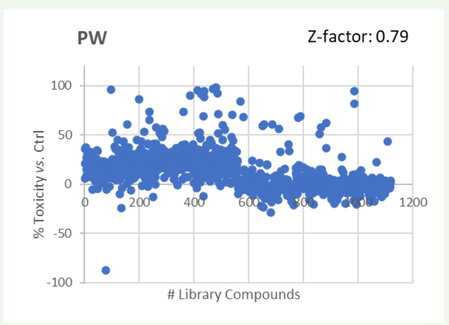
- ✓ Identify optimal combinations of chemotherapeutic drugs with the ALK inhibitor crizotinib (CRZ) in 2D and 3D GBM models.
- ✓ Employ selected combination to develop novel nanotherapeutics[5] that support targeted and controlled delivery in GBM patients.

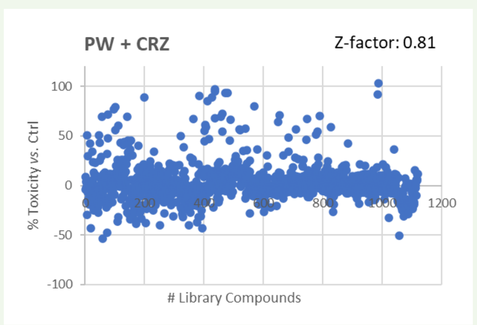
RESULTS & METHODS

1. High-throughput drug combination screening with CRZ

We subjected the GBM-derived cell line U87MG to a high throughput screening (HTS) of combinations of CRZ with the Prestwick library (PW), comprising 1120 FDA-approved drugs. We treated cells for 72 h with 2.5 μM of PW library compounds alone or in combination with the IC_{25} of CRZ (3.5 μM) (identified in previous studies (data not shown)). PW compounds were automatically added with LiHa Freedom Evo® (Tecan) and cytotoxicity was evaluated using MTS assays. The synergy scores were calculated using the Bliss synergy model. The HTS rendered a list of 14 potential compounds that synergized with CRZ according to the established criteria. Among them, we selected Hit 13 and performed dose-response curves to confirm the synergy in U87MG cells and two additional GBM cell lines, LN18 and A172 (data not shown).

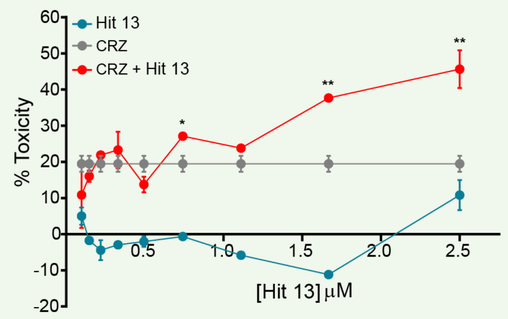






Compound	% Toxicity PW	% Toxicity PW+CRZ	Bliss Model Score
Hit 1	9	64	30.22
Hit 2	-5	64	40.93
Hit 3	-3	59	33.85
Hit 4	15	78	39.79
Hit 5	18	68	33.72
Hit 6	1	65	42.17
Hit 7	13	70	37.54
Hit 8	13	68	35.84
Hit 9	3	76	52.02
Hit 10	27	79	35.80
Hit 11	10	65	36.84
Hit 12	34	70	30.05
Hit 13	**16**	**56**	**32.00**
Hit 14	7	63	47.02

Selection criteria:
- Bliss score ≥ 30
- Toxicity PW + CRZ ≥ 50 %



[Hit 13] μM	0.098	0.15	0.22	0.33	0.49	0.74	1.11	1.67	2.50
Bliss score	-12.65	-2.03	6.00	7.17	-4.05	8.18	9.03	27.22	17.51

Antagonist			Synergistic	
<0	0 to 5	5 to 20	20 to 30	>30

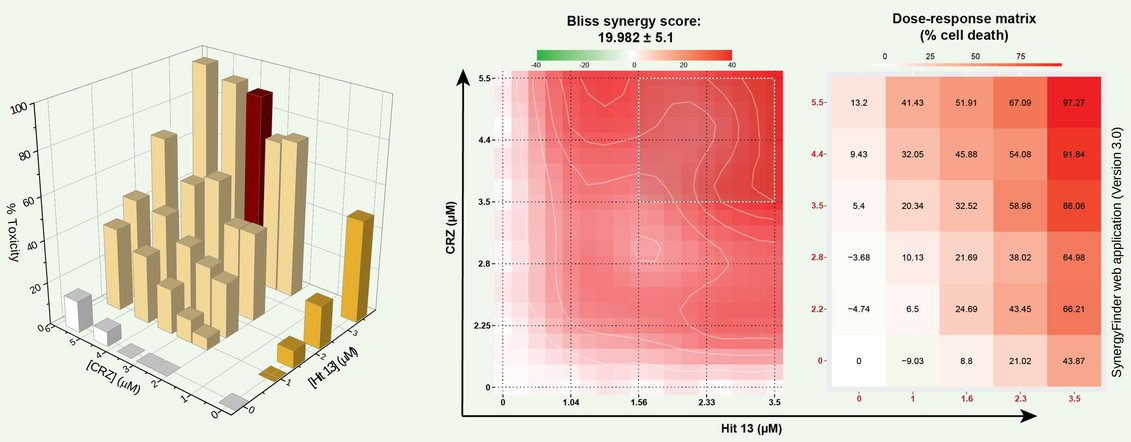
Bliss model

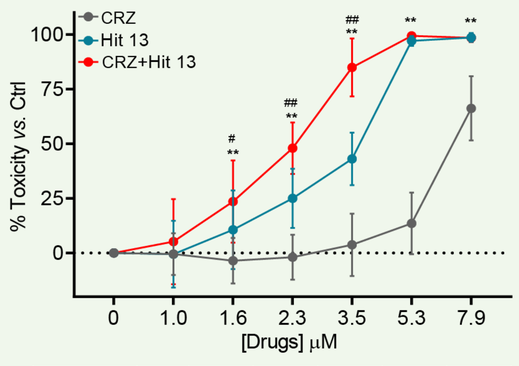
Observed response – Expected response

Expected response= rX(i)+ rY(j) - rX(i) rY(j)
rx(i)= % cell death with a dose i of a drug X
rY(j)= % cell death with a dose j of a drug Y

2. Optimization of the combination CRZ + Hit 13

U87MG cell treatment with different concentrations of CRZ and Hit 13 for 72 h and analysis with SynergyFinder highlighted a combination ratio of 1:1 at 3.5 μM as the optimal condition for synergy. We confirmed results in LN18 and A172 cells (data not shown).

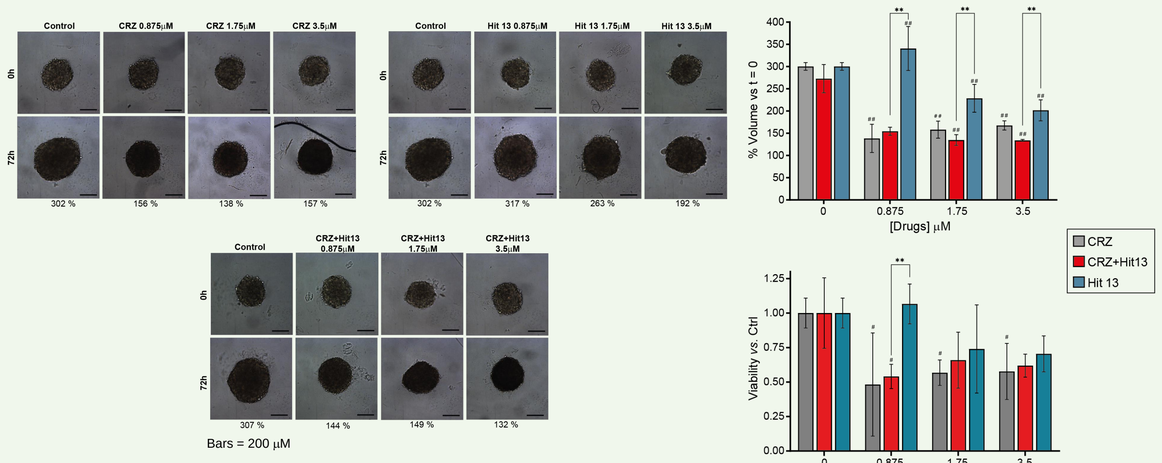


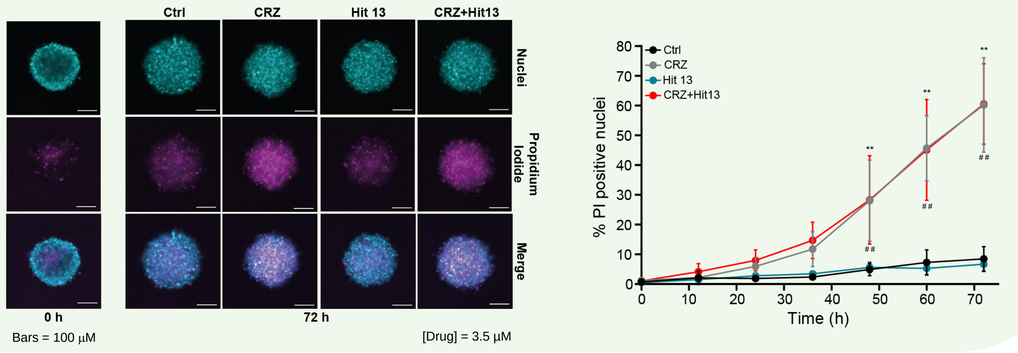


[Drugs] μM (Ratio 1:1)	1	1.6	2.3	3.5	5.3	7.9
Bliss score	6.21	16.03	24.37	39.72	1.92	-1.04

3. Combination CRZ + Hit 13 in GBM 3D models

We cultured U87MG spheroids in ULA-U-shaped 96-well plates for 72 h before treatment for 72 h. We determined cytotoxicity by ATP and by analyzing spheroid volume and cell death with propidium iodide staining with the Biotek Cytation 5 (Agilent). We treated spheroids with CRZ, Hit 13 or the combination (1:1). Surprisingly, CRZ displayed greater toxicity than expected and so we observed only small differences in the toxicity of CRZ and CRZ + Hit 13 in 3D. We hypothesize that spheroids better recapitulate the stemness properties of GBM, which would strengthen ALK signaling. Further experiments are needed to corroborate this hypothesis.





CONCLUSIONS & OUTLOOK

We described a list of potential compounds that increase the anti-tumoral effect of CRZ in GBM. Among them, Hit 13 emerged as the most suitable candidate for exploration as a component of novel combination nanotherapeutics for GBM treatment; however, further studies need to be performed to establish proper concentrations of CRZ:Hit13 in more complex models of GBM, such as spheroids, and to shed light on the molecular mechanism triggered by this combination.

REFERENCES
1. Louis, D. N. *et al. Acta Neuropathologica* **131**, 803–820 (2016)
2. Nieder, C., *et al. Critical Reviews in Oncology/Hematology* **60**, 181–193 (2006)
3. Kim, J.H. *Journal of Pathology and Translational Medicine* **55**, 236-237 (2021)
4. Allouche M. *Cell Cycle* **6**, 1533–1538 (2007)
5. Rodriguez-Otormin, F., *et al*. *Wiley Inter. Reviews: Nanomedicine and Nanobiotechnology* **11** (2019)

ACKNOWLEDGEMENTS
APOSTD/2021/239
PID2019-108806RB-I00