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# Validation of atypical drug combinations identified from combination databases

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## Background

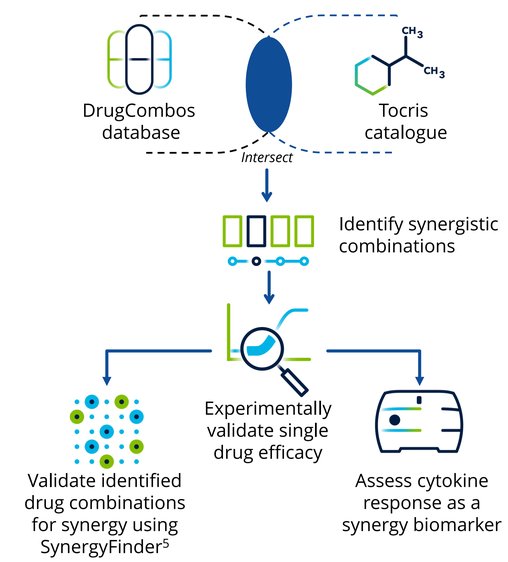
Rational drug combinations, based either on pharmacological or biological interactions, are being increasingly used in a clinical context, with many effective treatments using combination therapies. Implementing beneficial drug combinations can overcome clinical challenges including dose toxicity, drug resistance and lead to improved therapeutic responses[1]. Drug interactions can be classified into several types:

- Synergism – Combined effect is greater than the sum of effects seen with individual treatments
- Additivity – Observed response is the sum of the individual responses
- Potentiation – One drug does not elicit a response but enhances the effect of another drug
- Antagonistic – Exposure to one compound reduces the effect of the other

Identifying rational combinations with a clinically beneficial effect is a challenge, with the initial identification and validation based on preclinical work within cells and animal models[2]. Towards this, several data portals including DrugComb[3] portal and DrugcombDB[4] have come online to consolidate the results of cell-line-based drug combination studies. Providing a wealth of information, these comprehensive datasets allow for the identification of synergistic combinations across different tumour types and cell lines.

By intersecting commercially available drugs from the Tocris Catalogue with the DrugComb portal database, we set out to identify and interrogate atypical drug combinations. Focusing on combinations with the JAK inhibitor Ruxolitinib, an FDA-approved API with multiple indication including blood cancer, we experimentally validate atypical drug combinations reported as synergistic within the Lymphoma cell line, L-1236, and explore use of cytokines as a biomarker for synergy.

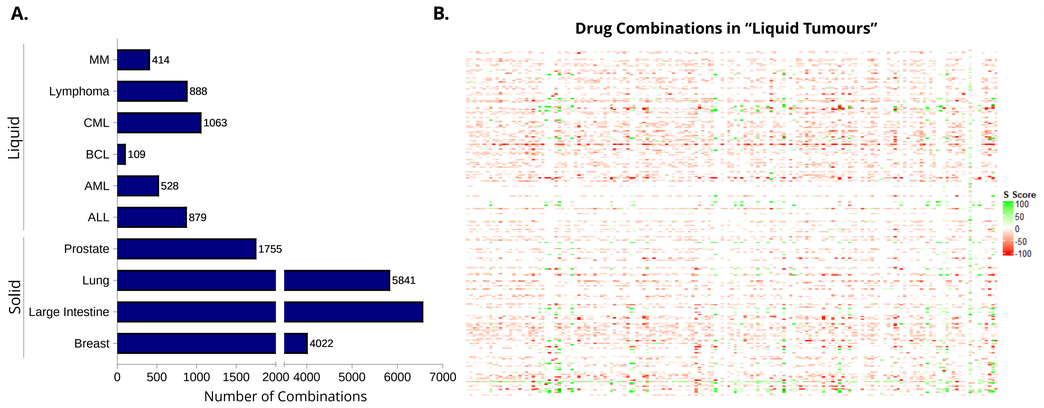
## Workflow Outline




## Results

### 1. Data intersection and identification of atypical synergies

Based on drug names, we successfully intersected publicly available data from the DrugComb database with commercially available small molecules available within the Tocris catalogue. By including cancer type, cell lines and synergy scores, a comprehensive dataset was generated, allowing for the exploration of known combinations across multiple cancers, cell lines and drug targets. Focusing on combinations with Ruxolitinib multiple atypical combinations with strong synergy are apparent, **Figure 1.**




| Drug Target | Cat.No |
|---|---|
| SV2A Modulator | 2839 |
| MAPK7, BMK1, ERK5, BRD4 Inhibitor | 4132 |
| CCR5 Antagonist | 3756 |
| BCL-2 Inhibitor | 6960 |
| IGF-1R Inhibitor | 7652 |
| PDGFR Inhibitor | 5906 |
| EGFR Inhibitor | 6811 |
| RAF Kinase | 6814 |
| VEGFR | 7454 |
| Wee1 | 7589 |
| PI-3K | 6792 |
| RAF Kinase | 4453 |
| MET Receptor | 5356 |

QR Code For Data Access:

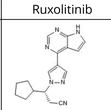
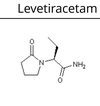
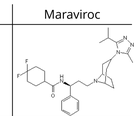
**Figure 1.** (A.) Number of interrogatable drug combinations by tumour type. (B.) Refined drug combination S Scores within "Liquid tumours". (C.) Defined list of combinations with Ruxolitinib (Cat No: 7064) in L-1236 cells with indicated S and BLISS synergy score in addition to pharmacological annotation of drug targets.

### 2. *In-cellulo* validation of drug combinations and cytokine release as a biomarker for potential synergy

Using cellular proliferation assays, we set out to validate the unexpected combination of Levetiracetam and Maraviroc with Ruxolitinib. Single-drug dose response shows that Ruxolitinib is potent as a single treatment, matching known IC50s for the L-1236 cell line. However, treatments with Levetiracetam and Maraviroc show no inhibition, with combined treatments with Ruxolitinib showing no observed synergy experimentally. Whilst testing dose responses, we set out to measure cytokine levels with the idea of using cytokine response as an early synergy biomarker prior to downstream viability effects, using a Luminex panel assay to identify correlative cytokines. Focusing on IL-6 response using the Ella platform and precisely tracking cytokine response to combined treatments, we observe an enhanced response following Ruxolitinib and Maraviroc treatment. **Figure 2.**

**A. Drug Profile and Synergy Summary**

**Overview**

| Drug Names | Ruxolitinib | Levetiracetam | Maraviroc |
|---|---|---|---|
| Structure |  |  |  |
| Target | Janus Kinase 1/2 (JAK1/2) | Synaptic Vesicle Protein A S(VPA) | C-C Chemokine Receptor type 5 (CCR5) |
| Type | Inhibitor | Modulator | Antagonist |
| Indication | Neoplasms | Epilepsy | HIV Infections |
| Approval Phase | Approved | | |

**Observed**

| Drug 1 | Ruxolitinib | | | |
|---|---|---|---|---|
| Drug 2 | Levetiracetam | | Maraviroc | |
| Bliss Score: | 107 | | 89 | |
| Reported Synergy Dosage (μM) | Drug 1 | 0.19 | Drug 1 | 0.14 |
| | Drug 2 | 0.07 | Drug 2 | 0.10 |

**Validation**

| | Drug Name: | IC50 (μM) | Observation |
|---|---|---|---|
| Single Dose | Ruxolitinib | 0.68 | - |
| | Levetiracetam | - | No effect on viability at 100μM |
| | Maraviroc | - | Minor decrease in viability at 100μM – likely cytotoxicity |

| Drug 1 | Ruxolitinib | |
|---|---|---|
| Drug 2 | Levetiracetam | Maraviroc |
| Bliss Score (Synergy Finder) | -1.42 | -1.39 |

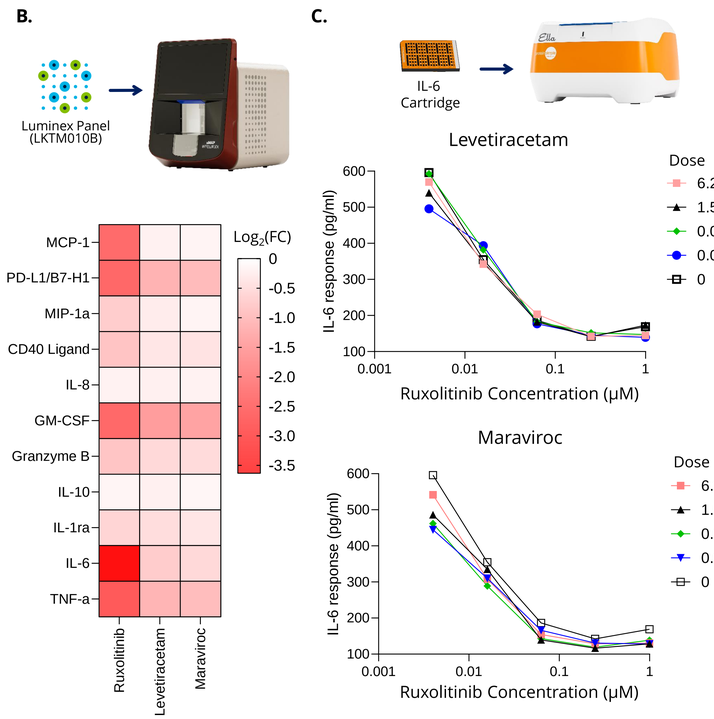



**Figure 2.** (A.) Summary for single drug and combination dose response for Ruxolitinib, Levetiracetam and Maraviroc and validation in L-1236 cells. (B.) Luminex instrument and heatmap showing fold change in cytokine response following, single drug treatment at 1 μM. (C.) IL-6 cytokine responses from Ruxolitinib treatment with Levetiracetam or Maraviroc using the Ella instrument.

## Conclusion

Integrating drug synergy data and available compounds provides an extensive knowledge base of assessable drug combinations for oncology and other indications. Whilst highly synergistic rational combinations are apparent in this data, several atypical combinations with reported synergies are observed despite these combinations lacking biological or pharmacological rationale. Direct biological validation of these atypical combinations does not recapitulate the reported synergy. However, by using cytokine readouts as a synergy biomarker, differential responses are observed suggesting a potentiating interaction.

In conclusion, combinations lacking an obvious pharmacological or biological rational, require direct biological validated against reported. Further to this, cytokine response is demonstrated as a potential biomarker for drug combinations.

**References:**

1. Wang and Bernards, 2022 *PMID: 36509911*
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3. Zagidullin et al. 2020 *PMID ID: 31066443*
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