Poster #117

# Combination Therapy in Melanoma:
## Finding Biomarkers of Synergistic Associations
using
## Large Scale Drug Combination Screening and Integrated Omics Data Analysis

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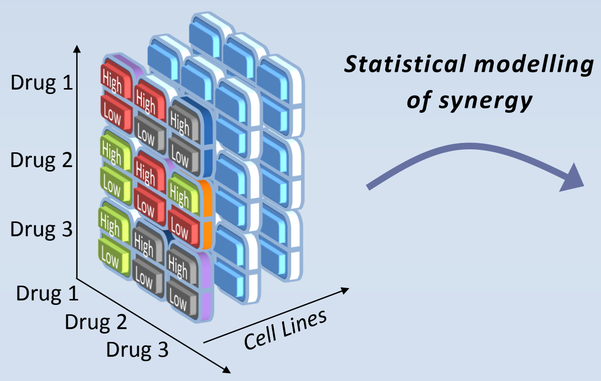
## Introduction

The lack of complete response and the emergence of resistance in large numbers of patients are pushing clinicians to search for combination therapies to prevent disease progression. The ability to perform large scale omic analysis against a large number of drugs is an opportunity to develop a systematic approach for identifying optimal drug combinations in preclinical settings that can be further validated in clinical trials.
Prediction of synergy of drug combination usually involves measurements of single drug effects[1,2] in comparison to the effect of the combination. The number of contexts (cell lines, etc.) is usually limited[1,3]. For each cell line, a heavy experimental workload is required[1,4] to obtain dose-response curves and transcriptomics data in different pharmacological contexts. Thus, synergy prediction using data from multiple, untreated (e.g. without drug administration) cell lines or tumor samples is highly beneficial from a clinical point of view.

## 1 • Experimental workflow

24 Melanoma cell lines | 108 Drugs | Low & high concentrations | 5778 Drug pairs combinations | Synergy : Bliss independence



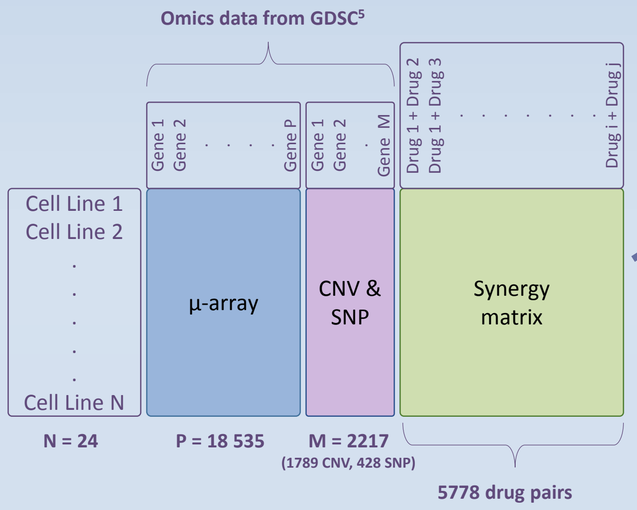

*Experimental data cube*
*108 x 108 x 24 x 2*

***Statistical modelling of synergy***

| cell.line | ABT-263_CHIR-265 | gemcitabine_AZD-7762 |
|---|---|---|
| A101D | synergistic | synergistic |
| A2058 | synergistic | synergistic |
| A375 | neutral | neutral |
| C32 | synergistic | synergistic |
| COLO792 | synergistic | synergistic |
| COLO800 | neutral | neutral |
| COLO829 | synergistic | neutral |
| G361 | synergistic | synergistic |
| IPC-298 | neutral | synergistic |
| IST-MEL1 | antagonistic | synergistic |

*Synergy matrix*
*24 x 5778*

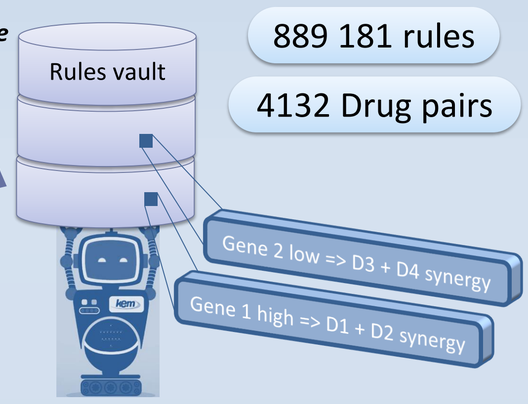
***Adding omics data***




*Analysis database*

***Exhaustive rule extraction with KEM®***

**Rules metrics assessment & filtering**




*KEM® BigData Rules management system*

### Statistical modelling of synergy

*The synergy is assessed with a Z-score (that reflects the deviation from the additive model) and P-value (that reflects the amount of noise)*

*Median polish*

$$viab^c_{i,j} = \frac{Nuclei^c_{i,j}}{\langle Nuclei^c_{DMSO,DMSO} \rangle}$$

*Linear model*

$$\log(viab^c_{i,j}) \sim \log(viab^c_i) + \log(viab^c_j)$$

Nuclei counts → Nuclei counts *(polished)* → Viability *(polished)* → Fit, residuals *(polished)*

|Residual| > noise ? → Synergy or antagonism

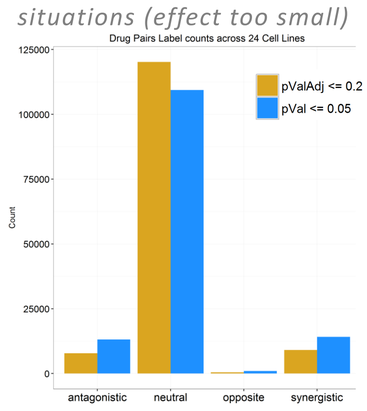
*Standard deviation in controls wells*

$$zval^c_{i,j} = \frac{Residual^c_{i,j}}{noise}$$ — *Normal distribution* → $pva^c_{i,j}$

*Synergy label is set in agreement with synergy scores at both concentrations: in case of disagreement, the label 'opposite' is used*

### P-value threshold

*The P-value threshold is chosen to optimize the trade-off between opposite situations (too much noise) and neutral situations (effect too small)*




### Associations Rules: definition, metrics

*KEM® generates association rules $Var_i \rightarrow Var_j$ in an exhaustive manner. These rules are characterized by 4 metrics that help ranking them.*

| | Var 1 | Var 2 | Var 3 (Endpoint) |
|---|---|---|---|
| Cell Line 1 | low | | 1 |
| Cell Line 2 | low | | 1 |
| Cell Line 3 | low | | 1 |

*Rule* Var 1 = low → Var 3 = 1

*Metrics*: Support | Confidence | Lift | P-value

**Support**: *number of times that the rule is checked in the dataset*

**Confidence**: *proportion of cases verifying Var1 = low that also verifies Var3 = 1.*

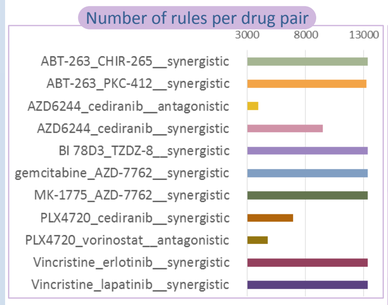
**Lift**: *ratio of the observed support to that expected if Var1 = low and Var3 = 1 were independent.*

**P-value**: *Fisher exact test*

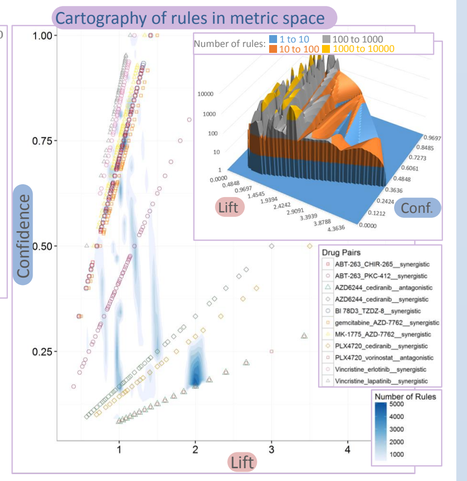
### Associations Rules: filtering and exploration

*Example with the 10 pairs in* Friedman *et al*[6]*. 118 290 rules were generated at relaxed filtering levels (Lift > 0.1 ; Confidence > 1% ; Support ≥ 2).*

Number of rules per drug pair




Cartography of rules in metric space

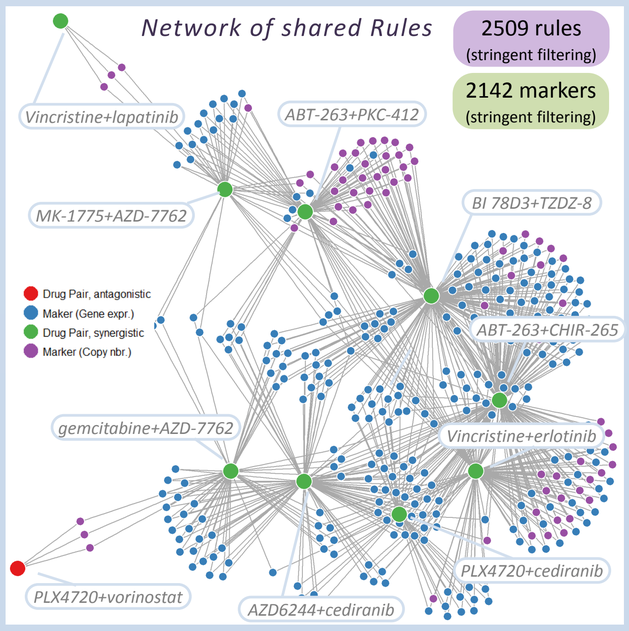



*In a second step, more stringent filtering applied to rules; high 'peaks' of rules correspond to rules that have the same metrics values.*

## 3 • Results

### 10 Drug pairs experimentally tested (see[6])

| DrugPairs | Syn | Antago | Neutral | Opposite | #Syn - #Antago Diff |
|---|---|---|---|---|---|
| Vincristine+lapatinib | 414 | 0 | 54 | 12 | 414 |
| BI 78D3+TZDZ-8 | 370 | 3 | 104 | 3 | 367 |
| Vincristine+erlotinib | 358 | 8 | 108 | 6 | 350 |
| ABT-263+CHIR-265 | 352 | 12 | 103 | 13 | 340 |
| gemcitabine+AZD-7762 | 326 | 6 | 139 | 9 | 320 |
| MK-1775+AZD-7762 | 326 | 11 | 125 | 18 | 315 |
| ABT-263+PKC-412 | 221 | 20 | 204 | 35 | 201 |
| PLX4720+cediranib | 128 | 5 | 279 | 8 | 123 |
| AZD6244+cediranib | 108 | 62 | 303 | 7 | 46 |
| PLX4720+vorinostat | 22 | 79 | 365 | 14 | -57 |




### New drugs combinations

PLX4720 (Vemurafenib precursor)

104 Drug pairs | Stringent filtering

Support >6 | Lift >1.3 | pval < 0.05

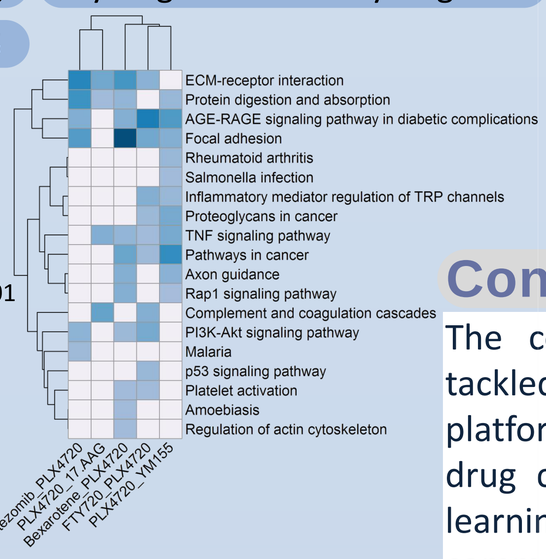
| Vemurafenib with | syn | antago | Diff | Trials |
|---|---|---|---|---|
| Bexarotene | 151 | 19 | 132 | no |
| FTY720 | 210 | 104 | 106 | no |
| YM155 | 134 | 67 | 67 | no |
| Bortezomib | 163 | 125 | 38 | NCT02788201 |
| 17.AAG | 93 | 108 | -15 | no |

*FTY720: Fingolimod*
*17.AAG: Tanespimycin*
*YM155: Sepantronium Bromide*

### Molecular mechanisms

Genes differentially expressed

Synergistic VS non-synergistic




*For each drug pair, the most differentially expressed genes between synergistic and non-synergistic cell lines are assessed. Corresponding KEGG annotation for the given drug pair shows that synergies target pathways in a cell-specific manner, but with commonly altered pathways, due to presence of vemurafenib in each drug pair.*

### Clinical relevance of synergy

clinicaltrials.gov | Phase II or higher | DCDB[7]

| DrugPairs | #Syn - #Antago Diff | Trial | Efficacy | Comments |
|---|---|---|---|---|
| Docetaxel+erlotinib | 211 | NCT00835471 | Good | OS improvement: 5.5 to 9.1 (months) |
| Bortezomib+vorinostat | 140 | NCT00773747 | Good | significant PFS improvement |
| Bortezomib+lenalidomide | 50 | UMIN8236 | Good | CR 43.8% |
| decitabine+temozolomide | 19 | NCT00715793 | Acceptable | ORR 18%, DCR 61% |
| sorafenib+temozolomide | 15 | NCT00811759 | Unknown | |
| sunitinib+sorafenib | 4 | NCT00732914 | Unknown | |
| Docetaxel+gemcitabine | -100 | NCT00236899 | Poor | not better than Paclitaxel |
| Docetaxel+YM155 | -161 | NCT01009775 | Poor | small improvement in ORR (12%) |
| AZD6244+Docetaxel | -200 | NCT01256359 | Poor | no significant improvement in PFS |

## Conclusion

The complex problem of drug synergy prediction is tackled here in a systematic way. The KEM® BigData platform allows us to extract omics markers for numerous drug combinations through a highly scalable machine-learning approach. The process allowed us to identify common markers shared across multiple drug pairs as well as specific ones. Moreover, the analysis of results from existing clinical trials on formerly identified drug pairs strengthens our confidence in the candidate combinations identified as synergistic and not yet in clinical development.

Although molecular mechanisms driving synergy are still unclear, identification of synergistic drug pairs and associated specific biomarkers may be transformed in the future into a therapeutic decision support system, suggesting optimal combination therapies for melanoma patients.

## References

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**6.** Friedman, A. A. *et al.* Landscape of Targeted Anti-Cancer Drug Synergies in Melanoma Identifies a Novel BRAF-VEGFR/PDGFR Combination Treatment. *PLOS ONE* **10,** e0140310 (2015).
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