Analysis of the combinatorial antiproliferative effect of pan RAF inhibitor AZ-628 and MEK1 inhibitor AZD-6244 (Selumetinib) on a large panel of tumor cell lines

Alokta Chakrabarti, Daniel Feger, Sarah Umber, Orysya Stus, Marianne Birkle, Oliver Siedentopf, Jan Ehlert

Reaction Biology Europe GmbH; Freiburg; Germany

1. Introduction

Cancer is a highly complex, multigenic disease with tumor cells underlying constant transition. Single drug treatments against specific targets frequently result in only partial success because mutations and redundant pathways cause drug resistance. Therefore, drug combinations that effect different synergistically acting targets in the cancer cell in parallel have become a promising strategy to improve the success in many fields of cancer therapy. One example for such an approach is the co-treatment of B-Raf driven tumors with Raf and MEK1 inhibitors.

In our study, we determined the combinatorial effect of the pan-Raf inhibitor AZ-628 and the MEK1 inhibitor AZD-6244 (Selumetinib) on the viability of a large panel of 120 tumor cell lines. Whittaker et al. (Mol Cancer Ther 2015) could already show that this drug combination has a significant synergistic effect in several melanoma and colon cancer cell lines. We here applied the combination of Selumetinib and AZ-628 in a broad checkerboard pattern to a multitude of cell lines from other entities beyond melanoma and colorectal cancer. Based on these results we correlated the observed synergistic and non-synergistic effects with gene expression profiles of these cell lines and analyzed the requirement of a MAPK activating signaling. Our approach revealed that synergistic activity is not confined to melanoma and colon cancer but is observed in tumors from other entities as well. This observation may expand the usefulness of MEK/Raf inhibitor co-treatment to a larger panel of cancer types.

2. Methods

Cell viability testing

Cells were seeded in white cell culture-treated flat and clear bottom multiwell plates and incubated at 37 °C o.N. before compounds were added. Compound treatment of cells started one day after seeding with a final DMSO concentration of 0.1% and was performed by nanodrop-dispensing using a Tecan Dispenser. 0.1% DMSO (solvent) and Staurosporine (1.0E-05 M) served as High control (100% viability) and Low control (0% viability), respectively. Compounds were added to the cells in a checkerboard pattern as below.

AZD-6244 (Selumetinib) [M]

AZ-628 [M]	1.00E-05	3.00E-06	1.00E-06	3.00E-07	1.00E-07	3.00E-08	1.00E-08	0
0								
1.00E-09								
3.00E-09								
1.00E-08								
3.00E-08								
1.00E-07								
3.00E-07								
1.00E-06								

Figure 1: Checkerboard scheme for addition of AZ-628 and Selumetinib to cells.

After incubation for 72 h at 37°C at 5% or 10% CO2 dependent on the medium, cell plates were equilibrated to room temperature for one hour, CellTiterGlo reagent (Promega) was added and luminescence was measured an hour later using a luminometer. The IC50 was determined using the sigmoidal slope regression in the software GraphPad Prism 5. Synergy was determined using Bliss independence model. A variation of ±0.1 was accepted.

Data analysis

1. Examination of results by Bliss-Factor analysis:

Taking the effects observed at the different concentrations of the compounds alone, a Bliss-Factor matrix was used [E1+E2=E1+E2-E1xE2] to calculate the expected effects for a merely additive situation. This was compared with the actual data obtained. The difference of both numbers is given in the Bliss factor analysis, showing positive numbers for synergistic and negative numbers for antagonistic effects. These calculations are shown as three-dimensional plot: a hill on the plot indicates synergism, a valley antagonism.

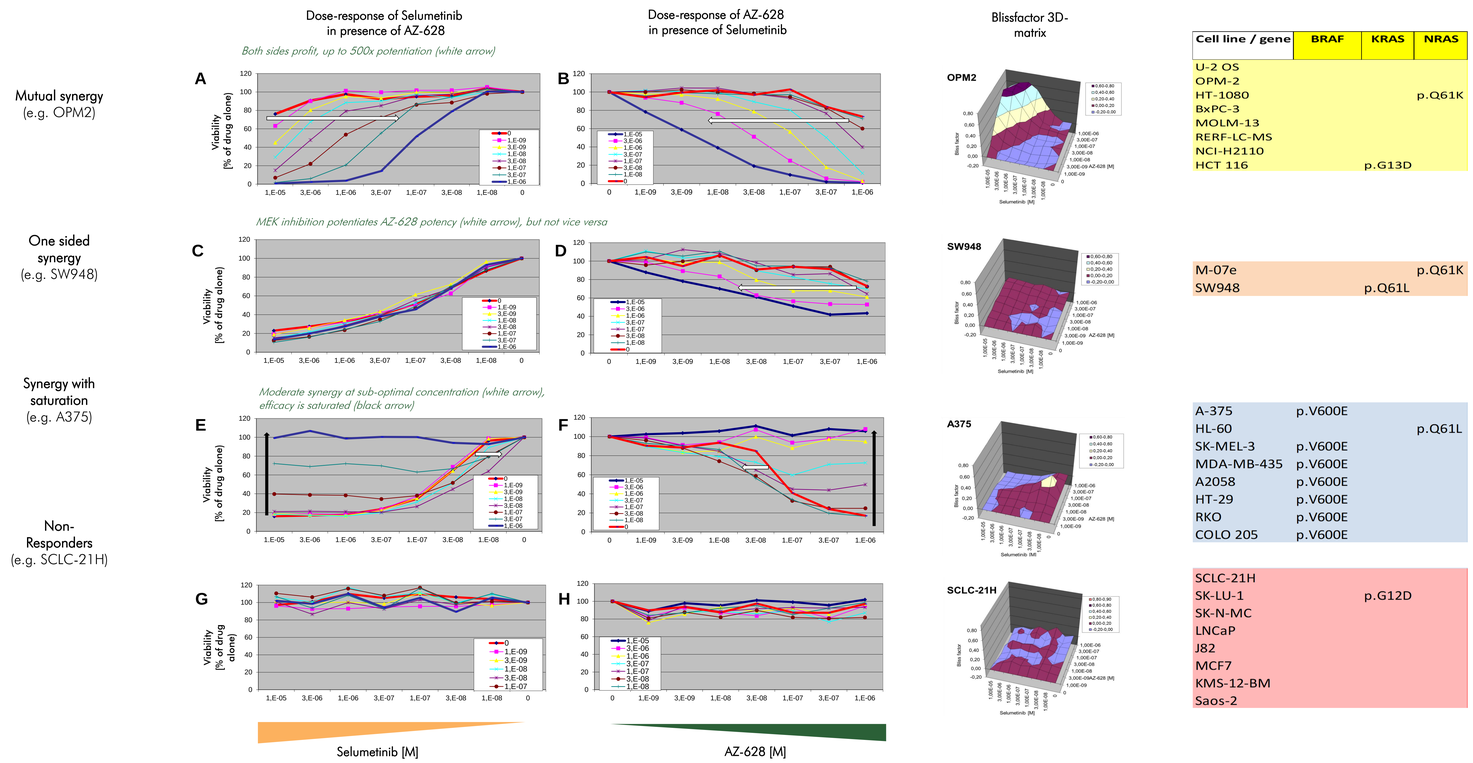
2. Comparison of dose-response curves of the respective first compound with and without the different concentrations of the second compound.

To that end, counts obtained for the second compound alone were set to a new High value (100%) and data obtained at different concentrations of the first compound were set in relation to that High value. An additive combination would result in similar overlapping dose-response curves as compared to the curve obtained with the first compound alone. A synergistic combination would improve the potency of the first compound with increasing amounts of the second compound and vice versa an antagonistic combination would reduce the potency.

Gene expression and mutation analysis

PharmacoGx, a R package for analysis of large pharmacogenomic datasets, was utilized to access normalized RNA expression data curated from the Genomics of Drug Sensitivity in Cancer (GDSC) and Cancer Cell Line Enclycopedia (CCLE) databases. The RNA expression data was filtered for null values and unlabeled RNA probes. Processed data was inputed into Tableau (version 9.3) for the development of interactive dashboards allowing easy, quick, accurate data visualizations and data access. Mutation data were curated from both databases.

3. Results



Cell line / gene	BRAF	KRAS	NRAS
U-2 OS			
OPM-2			
HT-1080			p.Q61K
BxPC-3			
MOLM-13			
RERF-LC-MS			
NCI-H2110			
HCT 116		p.G13D	
M-07e			p.Q61K
SW948		p.Q61L	
A-375	p.V600E		
HL-60			p.Q61L
SK-MEL-3	p.V600E		
MDA-MB-435	p.V600E		
A2058	p.V600E		
HT-29	p.V600E		
RKO	p.V600E		
COLO 205	p.V600E		
SCLC-21H			
SK-LU-1			p.G12D
SK-N-MC			
LNCaP			
J82			
MCF7			
KMS-12-BM			
Saos-2			

Impact of the Selumetinib/AZ-628 combination on the proliferation of 120 cell lines was analysed in dose-response curves of the compounds in the presence of the respective other compound looking at potentiation (figure A-H, legend shows conc of respective combination compound; **bold red line=** dose response alone; **bold blue line=** dose reponse of highest conc of combination agent). Four response types have been identified, for each of which results of one example cell line is shown, including the 3D-Blissfactor matrix. All cell lines showing significant such responses are listed with corresponding mutation status in BRaf, NRas and KRas. No mutations have been observed for HRas, nor any correlated expression levels.

4. Summary and Conclusion

- Selumetinib and AZ-628 have an excellent synergistic potential in many cell lines derived from various entities. In principal, three synergistic response types were observed:
- Mutual Synergy: In 8 of 120 cell lines, compound activity potentiation between 20 to 500fold was observed. This involved cells that hardly respond to one compound alone but which boost in the simultaneous presence of the other. These cells do not bear BRaf mutations nor, for the most part, KRas or NRas mutations.
- One-sided-synergy: A small subgroup of cells (2 of 120) shows one-sided synergy, i.e. MEK-inhibition by Selumetinib significantly potentiates proliferation inhibition by Raf-directed AZ-628, but not vice versa. Both cell lines bear activating Q61 mutations either in KRas or NRas.
- Synergy with saturation: Observed in 8 of 120 cell lines. Compounds in suboptimal concentrations show moderate synergy. At saturating concentrations mutual neutralization. Confined to cells that have mutated BRafV600E which are per se the most sensitive cells to both compounds alone. Interesting: Cell line HL60 sticks out as non Braf mutant!