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NOVEL DRUG COMBINATIONS FOR THE TREATMENT OF REFRACTORY TESTICULAR GERM CELL TUMORS IDENTIFIED BY MULTIPLEXED DRUG SCREENING

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STUDY RATIONALE: Testicular germ cell tumors (TGCTs) develop resistance to cisplatin therapy by pleiotropic mechanisms. Therefore, we assessed antiproliferative efficacy of 22 different clinical stage-inhibitors on 13 different cisplatin-resistant TGCT cell lines in order to identify novel clinical strategies for chemorefractory TGCTs patients.

STUDY DESIGN: We derived cisplatin-resistant embryonal carcinoma (EC) model cell lines from established **NCCIT**, **NTERA2** and **NEC8** cell lines. Moreover, we used previously derived platinum-resistant **NCCIT**, **2102Ep** and **TERA2** cells and inherently resistant **TERA1** cells provided as a kind gift from other laboratories. Seminoma **TCam2**, choriocarcinoma **JEG3**, **JAR,** yolk sac tumor **NOY1** and teratocarcinoma **SuSa** cell lines were also included.

Following SMIs were tested (target kinases indicated in brackets): **Adavosertib** (Wee1), **Tazemetostat** (EZH2), **Selinexor** (CRM1), **RO4929097** (gamma-secretase, Notch, Aβ40), **Ganetespib** (HSP90), **Simvastatin** (HMG-CoA reductase), **Entrectinib** (TrkA, TrkB, TrkC, Ros1, ALK), **Enasidenib** (mutIDH2), **Curcumin** (Nrf2, Ferroptosis, HDAC), **Roxadustad** (HIF propylhydrolase), **Cyclosporin A** (calcineurin), **Dasatinib** (Abl, Src, c-Kit(D816V), c-Kit(wt)), **Ruxolitinib** (JAK1, JAK2), **Copanlisib** (PI3K αβγ), **ABBV-744** (BDII), **Devimistat** (PDH, α-KGDH), **Fenofibrate** (PPAR-α, CZP2C19, CYP2B6), **Vismodegib** (Hedgehog), **Crizotinib** (ROS1,C-Met), **Tozasertib** (Aurora A, Aurora B, Aurora C, FLT3, Bcr-Abl), **Galunisertib** (TβRI), and **Plurodemstatbesylat** (LSD1). SMIs were used as a single agent or in combination with cisplatin. We identified high antiproliferative activity of Ganetespib, HSP90 inhibitor, Adavosertib, WEE1 inhibitor, and Selinexor as a selective inhibitor of nuclear export, that inhibits exportin-1 protein (XPO1). Moreover, dasatinib exerted synergistic effect with cisplatin in some platinum refractory TGCT cell lines. The data were validated by three independent methods of cytotoxicity measurement based on luminescence, live-cell kinetic imaging and kinetic label-free impedance platform Maestro Z system (Axion BioSystems). Data validation for the combinatorial effects of other SMIs is ongoing.

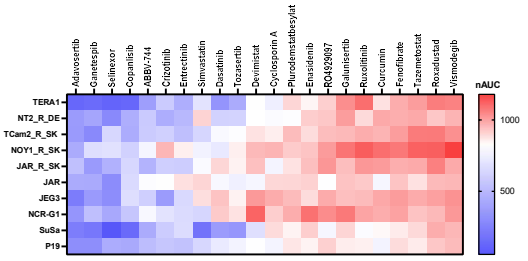
Drug screening workflow

STEP 1
Drug screen
22 drugs

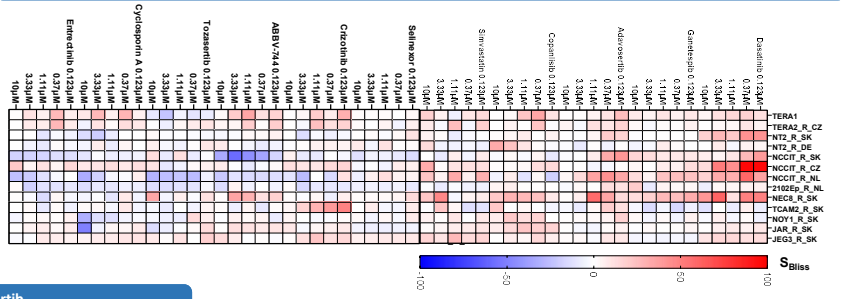
STEP 2
Combination drug screen
11 pre-selected compounds
13 cisplatin-resistant cell lines
→ synergy, additive effect vs. antagonism

STEP 3
Validation
→ endpoint luminescence viability
→ multiplexed impedance/luminescence
→ multiplexed image-analysis/luminescence
→ 3D spheroid kinetic/endpoint luminescence

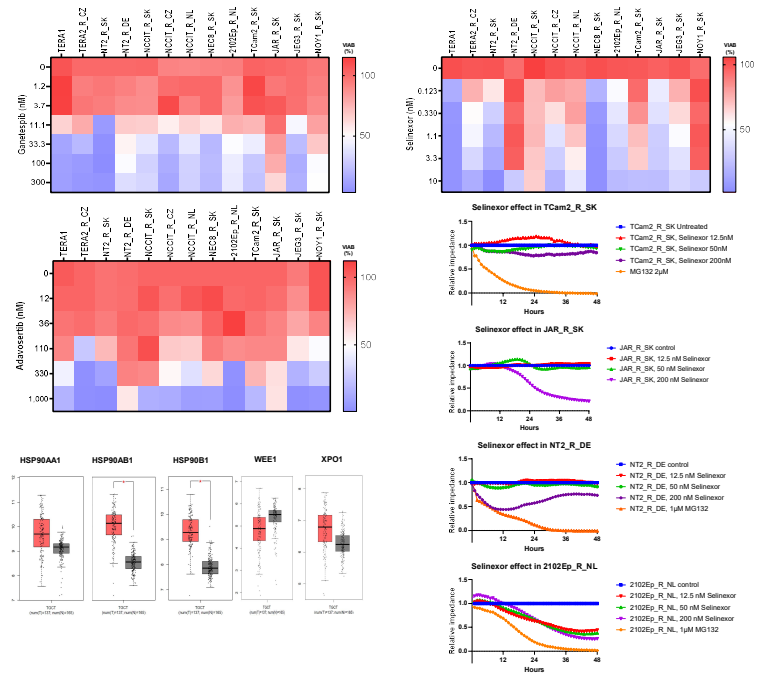
Antiproliferative effect of small molecule inhibitors of intracellular signalling used as a single agent in selected TGCT cell lines



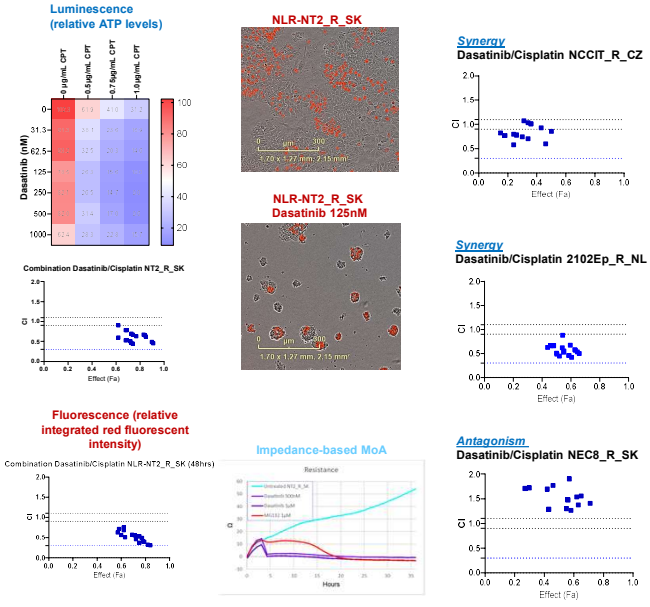
Antiproliferative effect of selected small molecule inhibitors of intracellular signalling used in combination with cisplatin in selected chemoresistant TGCT cell lines



Antiproliferative effect of ganetespib, selinexor and adavosertib used as a single agent in cisplatin resistant TGCT cell lines



Validation of combination effect of dasatinib and cisplatin used as a treatment in cisplatin-resistant TGCT cells



Conclusion. New molecules emerge as potential agents to treat cisplatin-resistant germ cell tumors either as single treatment or in combination with cisplatin in synergistic fashion. Our broad panel of chemoresistant TGCT cell lines enables high-throughput screening of compounds that could rapidly enter clinical testing.

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