oncogenesis. Therefore, TPX-0005 could be a promising novel therapeutic option for patients with *ALK*-mutant neuroblastoma.

Methods: *In vitro* sensitivity to ALK inhibitors (TPX-0005 and ensartinib) and cytotoxic chemotherapy, singly or in combination, were evaluated in neuroblastoma cell lines harboring *ALK* mutations and with variable *MYCN* status. *In vivo* anti-tumor effect of TPX-0005 monotherapy, and in combination with chemotherapy, was evaluated using pediatric patient derived xenograft (PDX) models of neuroblastoma and an *NTRK*-fusion positive solid tumor model.

Results: Treatment of three neuroblastoma cell lines demonstrates that TPX-0005 has cytotoxic activity across *ALK*-mutant and *ALK*-WT cell lines (with IC_{50} 2-3X lower in *ALK*-mutant lines). Combination of ALK inhibition (TPX-0005 or ensartinib) with irinotecan and temozolomide demonstrated synergistic antiproliferative activity across cell lines. *In vivo* therapeutic studies evaluated anti-tumor effect and event-free survival of models treated with TPX-0005 compared to a comparator drug (ensartinib for neuroblastoma models and entrectinib for the *NTRK*-fusion solid tumor). TPX-0005 monotherapy treatment prolongs event-free survival ($p < 0.05$, log-rank test) and has notable anti-tumor effect ($p = 0.006$ versus vehicle and $p = 0.05$ versus comparator drug). Furthermore, TPX-0005 plus chemotherapy *in vivo* is superior to chemotherapy alone when comparing tumor growth curves in an *ALK*-mutant neuroblastoma model ($p = 0.025$, Vardi's test). TPX-0005 also induced a near complete response (>80% tumor volume reduction) after four weeks of treatment in a PDX model characterized by *ETV6-NTRK3* fusion harboring a known solvent front mutation.

Conclusions: TPX-0005 demonstrates anti-tumor activity in *ALK*-mutant tumor models singly and in combination with chemotherapy *in vivo*. A significant improvement in event-free survival across all models, and particularly when combined with chemotherapy in an *ALK*-mutant PDX model, provides preclinical rationale for further exploration of TPX-0005 in *ALK*-mutant pediatric tumors.

No conflict of interest.

35 Poster Discussion

High-throughput small molecule screens reveal therapeutic opportunities against TFE3-fusion renal cell carcinoma

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Background: Accounting for 1–5% of all renal cell carcinoma (RCC) cases, TFE3-fusion RCC comprise an aggressive subset of neoplasms typically diagnosed in early and middle adulthood, for which treatment options are currently scarce. We sought to describe the distinctive TFE3-fusion RCC transcriptomic landscape, to perform high-throughput drug screening and to validate potential therapeutic targets using a combination of *in vitro* and *in vivo* experimental approaches.

Material and methods: Seven TFE3-fusion RCC tissue samples were subjected to RNA sequencing and pathway analysis was performed on differentially expressed genes. High-throughput drug screening with 1912 clinically relevant compounds was carried out on 3 patient-derived TFE3-fusion RCC cell lines harboring different TFE3-fusion genes. Cell viability results were validated in 2D and 3D spheroid models of 5 TFE3-fusion RCC cell lines. Drug mechanism was assessed by evaluating cell cycle and apoptosis, transcriptional activity assays and mTOR pathway inhibition assays. Subcutaneous nude mice xenograft studies were carried out using 2 TFE3-fusion RCC cell lines. Combination drug studies were performed *in vitro* and *in vivo*.

Results: Transcriptomics analyses of human TFE3-fusion tumor tissues showed high expression of lysosomal-related genes and upregulation of transcriptional profiles driving cell cycle and chromosome segregation. These data were integrated into the drug prioritization process of the quantitative high-throughput small molecule screen. After *in vitro* validation of candidate small molecules, the *in vivo* efficacy of 5 compound classes, namely proteasome inhibitors, PI3K/mTOR inhibitors, SRC inhibitors, RNA synthesis and microtubule inhibitors was validated in xenograft studies. The SRC inhibitor Dasatinib, the RNA synthesis inhibitor Mithramycin and the PI3K/mTOR inhibitor NVP-BGT226 presented significant antitumor activities *in vivo* as single agents. Molecular analyses of the mechanism of action of each compound identified the importance of the activation the SRC pathway, SP1 transcription factor activation and the expression of survivin in the cell cycle progression and survival of TFE3-fusion RCC cell lines, and confirmed the role of the Akt/mTOR pathway in the growth of TFE3-fusion RCC cells. Combination drug studies of these compounds, together with a previously discovered drug target, the GPNMB-targeting antibody-drug-conjugate CDX-011, showed significant tumor inhibitory activities *in vitro* and *in vivo*.

Conclusions: PI3K/mTOR pathway inhibitors (NVP-BGT226), transcription inhibitors (Mithramycin), and combinations among each other and with the antibody-drug conjugate CDX-011, show promising preclinical efficacy against TFE3-fusion RCC. This preclinical study provides an unbiased foundation to identify potential therapeutic approaches against TFE3-fusion RCC patients.

No conflict of interest.

36 Poster Discussion

Screening of fractions from marine sponges and other invertebrates to identify new lead compounds with anti-tumor activity in lymphoma models

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Background: Diffuse large B-cell lymphoma (DLBCL) is the commonest type of lymphomas, accounting for 30%-40% of new cases each year. Despite the big improvements achieved in the treatment, still 25–40% of patients still succumb due to refractory or relapsed disease. This highlights the need of new drugs for this cancer. The marine environment has recently been recognized as a source of anti-cancer compounds, as demonstrated by different marine drugs approved by different regulatory agencies (trabectedin, cytarabine, eribulin, plitidepsin) or as components of antibody drug conjugates for lymphoma patients (monomethyl auristatin E in polatuzumab vedotin and brentuximab vedotin). Here, we present a large screening of fractions obtained from different marine invertebrates collected in Ireland and in the Pacific Ocean on DLBCL cell lines.

Material and methods: Marine invertebrate fractions were prepared by C18 Solid Phase extraction using solvents of different polarities and they were stored in DMSO at 10 mg/mL; Cells were seeded in 96 well plate with the semi-automatic dispenser INTEGA VIAFLO at the density of 10000 cells for each well. Cell lines were exposed to fractions for 72 hours at the concentration of 100 or 10 or 1 mg/ml followed by MTT assay, as previously performed (Spriano et al, CCR 2018).

Results: 583 fractions were tested in 2 DLBCL cell lines (TMD8 and OCI-LY10) and 1 non neoplastic lymphoblastoid B cell line (CB33), used as DLBCL normal counterpart. At the concentration of 100 mg/ml the majority of the fractions showed an anti-proliferative activity in both lymphoma cell lines, while at 10 mg/ml only around half of the fractions still showed such biologic activity. Conversely, only 20 fractions determined over 30% reduction of cell proliferation in both lymphoma cell lines when given at 1 mg/ml concentration. Moreover, among these 20 with antitumor activity, 5 showed no activity at all in the lymphoblastoid cell line; 4 fractions caused the same effect as in the lymphoma cell lines and 11 cause some reduction in proliferation but not as much as in the neoplastic models. Among the most promising fractions we identified those coming from the Irish ascidian *Diplosoma* listerianum.and the nudibranch *Janolus cristatus* but also the Pacific sponge *Stylissa* cf. *carteri*. Work is ongoing to identify the compounds responsible for the remarkable bioactivities.

Conclusions: In this first screening, we identified fractions derived from marine invertebrates with anti-tumor activity in lymphoma cell lines, including some without any effect on the proliferation of a non-tumoral B-cell model. These fractions provide the material to potentially identify novel anti-cancer compounds of marine origin. OT and FB are members of the COST Action CA18238 Ocean4Biotech.

No conflict of interest.