152 (PB032)

NUAK1 directly induces Akt signaling and substrate specificity, promoting cancer cell survival

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Background: NUAK1 is a serine/threonine kinase member of the AMPKα-family, whose high expression is associated with poor prognosis. However, NUAK1 regulation and functions in cancer remain poorly characterized. Here, we investigated NUAK1's role in cancer signaling.

Materials and Methods: We performed a bioinformatic analysis of TCGA to study the correlation between NUAK1 expression and the expression of EGFR and Akt phosphorylation in several cancers. We mainly performed the studies on breast and colon cancer cell lines. Pharmacological or shRNA-dependent inhibition of NUAK1 demonstrated its role in the EGFR and insulin-dependent signaling. In vitro kinase assays were performed to demonstrate the direct phosphorylation of Akt by NUAK1, and proximity ligation assays demonstrated the association of NUAK1 with Akt in cancer cells. Cell fractionation and immunofluorescence studies analyzed NUAK1 subcellular localization, and qPCR studies demonstrated its role in the Akt/FOXO1/3a axis. We used 2D and 3D cultures for cell survival studies and monitored cell death using commercial kits.

Results: Based on public TCGA data, we reported that NUAK1 expression correlates with EGFR expression and the Akt phosphorylation at Ser-473 in several cancers. Using pharmacological inhibition and shRNA-dependent silencing, we found that NUAK1 activates the Akt signaling, regulating FOXO1/3a and GSK3β phosphorylation but not TSC2 phosphorylation. Mechanistically, NUAK1 interacts with Akt and directly phosphorylates it at Ser-473. Comparing NUAK1 and mTOR inhibition revealed an Akt-dynamic activation and -substrate specificity depending on its phosphorylation by NUAK1 or mTORC2. The Akt-substrate specificity could be directly associated with NUAK1 co-localization with early endosomes. Functionally, the NUAK1/Akt/FOXO1/3a axis reduced p21CIP1 and p27KIP1 expression but induced FoxM1 expression. Additionally, our study identified that NUAK1 promotes cancer cell survival in a growth factor-dependent manner, and its inhibition potentiates the effect of MK-2206, an Akt inhibitor.

Conclusion: We demonstrated that NUAK1, in contrast to other AMPK-related members, regulates cancer signaling via direct Akt phosphorylation. Thus, targeting NUAK1, either alone or combined with Akt inhibitors, may be effective in cancers with hyperactivated Akt signaling.

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153 (PB033)

Using Tipifarnib to prevent resistance to targeted therapies in oncogene-addicted tumors

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Purpose: Targeted therapies can provide impressive responses in oncogene-addicted tumors, but are almost never curative due to the inevitable emergence of resistance. In this context, Drug-Tolerant Cells (DTC) have emerged as a new relevant concept which could explain the very first step of drug resistance, particularly in non-small cell lung cancers (NSCLC) and metastatic melanoma. We recently discovered that the farnesyltransferase inhibitor (FTi) tipifarnib can prevent the emergence of resistance to tyrosine kinase inhibitors in EGFR-mutant NSCLC *in vitro* and *in vivo*, by interfering with several key factors of the adaptive response, such as Rho GTPases and cell division-related proteins (Figarol *et al.*, BioRxiv 2022). Here, we report that co-treatment with tipifarnib can also prevent relapse to targeted therapies in other oncogenic settings such as *KRAS*-G12C and *ALK*-translocated NSCLC or *BRAF*-mutant melanoma, suggesting a common vulnerability of DTC to the FTI tipifarnib.

Experimental design: H3122 (EML4-ALK NSCLC), H23 and Calu-1 (KRAS-G12C NSCLC), and A375 (BRAF-V600E metastatic melanoma) cell lines were treated with 1 μM lorlatinib, sotorasib or dabrafenib, respectively, alone or in combination with 1 μM tipifarnib. Cells were previously transduced by the FUCCI (fluorescence ubiquitination cell cycle indicator) system to monitor the cell cycle dynamics in real time during the adaptive response. Signalling pathways affected by the treatments were determined by Western Blot. *in vivo* studies using KRAS-G12C- and EML4-ALK-derived NSCLC PDX are ongoing.

Results: All the cell lines tested displayed an initial response to their corresponding monotherapy, characterized by an accumulation in G1 within the first 48 h, followed by a more or less intense cell death depending on the cell line. Similar to what was observed in EGFR-TKi-treated NSCLC, some cells, referred to as "early escapers," could escape G1 and progress through S/G2 during the initial response phase, progressively giving rise to resistant proliferative clones. Co-treatment with tipifarnib prevented relapse in all the models tested by impairing mitosis of S/G2 early escapers and promoting apoptosis. As in EGFR-mutant models, response to the different monotherapies invariably involved activation of the p27/pRb pathway, RhoB overexpression, and formation of actin stress fibers, which appeared to be hallmarks of the drug-tolerant state most likely responsible for the susceptibility of DTC to tipifarnib.

Conclusion: Our data provide a strong biological rationale for the development of combinations with targeted therapies and tipifarnib in the clinic.

Conflict of interest:
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154 (PB034)

Identification of an effective chemotherapy and DNA damage response inhibitor combination for diffuse large b cell lymphoma

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Background: Chemotherapy forms the backbone of treatment for Diffuse Large B Cell Lymphoma (DLBCL); however, <~20% of tumors are chemoresistant. Inhibitors of the DNA damage response (DDR) show promise as chemosensitizers. We therefore set up an *in-vitro* screen to identify an optimal chemotherapy-DDR inhibitor (DDRi) combination in a panel of DLBCL cell lines.

Material and Methods: To achieve this, we harnessed Quadratic Phenotypic Optimization Platform (QPOP), an experimental-analytic method built to identify potent drug combinations. 6 DDRis (to ATR, ATM, CHK1/2, DNA-PK, WEE1, PARP) and 6 routinely used chemotherapy agents were selected for the screen. After identifying the most effective combination using a cell viability assay, we investigated the mechanism of synergy. We observed pathway activation by western blot; apoptosis by Annexin V staining; and cell cycle disruption by flow cytometry with EdU, Propidium Iodide, and phospho-Histone H3 staining. To explore the underlying mechanism of synergy, we performed RNA sequencing.

Results: From the combination screen, ATR inhibitor, AZD6738, and chemotherapeutic drug, Gemcitabine (A+G), was the most effective combination across multiple DLBCL cell lines, including Gemcitabine-resistant cell lines. Using two gemcitabine-resistant DLBCL cell lines as models, we investigated the mechanism of A+G synergy. Inhibition of ATR abrogates the G2M checkpoint, causing cells with gemcitabine-induced DNA damage to enter mitosis and die through mitotic catastrophe. We confirmed that the combination reduced cell viability, affected Chk1 phosphorylation,

and promoted apoptosis. However, only a small proportion of cells enter mitosis after A+G treatment and cells were primarily arrested in G1 phase with blockade of entry into S phase. Pathway analysis by RNA seq revealed that several cell-cycle and DNA replication-related pathways were suppressed in the combination setting. Interestingly, the transcriptome of A+G treated cells revealed a reversal of a gene expression signature characteristic of dark zone (DZ) biology. The DZ signature reflects a gene expression program associated with B-cells in the DZ of the germinal centre and is enriched in poorly prognostic DLBCL.

Conclusions: Taken together, we have identified a chemotherapy-DDRi drug combination, AZD6738 and Gemcitabine, which is effective in killing DLBCL cells *in-vitro*, including cells that are resistant to Gemcitabine. The mechanism of synergy is not likely to be through mitotic catastrophe, but may potentially involve a cell cycle state reflecting the suppression of a B-cell specific transcriptional program regulating the DZ-LZ transition. Importantly, the reversal of the DZ gene expression signature by the combination indicates its potential utility as a treatment option for these lymphomas that exhibit this poor-prognostic gene signature.

Conflict of interest:
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155 (PB035)

Derazantinib, an inhibitor of fibroblast growth factor receptors 1–3, increases the efficacy of paclitaxel combined with a VEGFR2-antibody in murine syngeneic tumor models

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Background: Derazantinib (DZB) is an oral fibroblast growth factor receptor (FGFR) inhibitor with clinical activity in intrahepatic cholangiocarcinoma. DZB is also in a phase-2 trial for gastric cancer (GC), where it is combined with the current standard-of-care (SoC) paclitaxel and the VEGFR2-antibody (Ab), ramucirumab. Kinase assays and cellular and in vivo data indicate significant activity of DZB against two other important targets in oncology, CSF1R and VEGFR2. Here, using three different syngeneic tumor models grown orthotopically or subcutaneously in mice, we have explored the efficacy and tolerability of combining DZB with paclitaxel and a murine VEGFR2-Ab.

Materials and Methods: Female Balb/c mice were used to host three different models: the breast tumors 4T1 and EMT6 both grown orthotopically in the mammary fat pad, and colon MC38 grown subcutaneously. When the mean tumor size was at least 80 mm^3, mice were treated with vehicles (po, ip and iv), DZB alone (35 or 75 mg/kg, po, qd), paclitaxel (15 mg/kg, iv, qw) or the VEGFR2-Ab, DC101 (10 mg/kg_3 ip, 2qw). Treatments continued until individual tumors reached 1500 mm^3, when mice were culled and tumors ablated for formalin-fixing and paraffin-embedding. Efficacy (ΔT/C) was determined a) by the change in tumor-volume at the day of first culling, and b) median time to 1500 mm^3 to allow longer treatment study in the very fast-growing models of EMT6 and MC38. The 4T1 model was stopped after 3-weeks to allow quantification of metastases by counting lung nodules. The combination interaction was assessed formally as synergy/additivity/antagonism by the Clarke-Combination-Index (CCI). Tolerability was assessed by the %-change in body-weight.

Results: MTD studies using non-tumor bearing mice over 3 weeks showed that half the full dose of DZB (35 mg/kg, po, qd) could be combined with the full doses of paclitaxel and DC101 (Triple-combination). In the 4T1 model, the Triple significantly increased efficacy compared to the SoC of paclitaxel-DC101 in the primary tumor (ΔT/Cs: 0.25 and 0.6 respectively) and also reduced the lung weight and metastases. In the EMT6 model, the Triple was more efficacious than the SoC against the primary tumor (ΔT/Cs: 0.42 and 0.86 respectively). The CCI for these breast tumor models indicated an additive effect, and the Triple also gave increased efficacy compared to the other two doublets (paclitaxel-DZB and DZB-Ab). The MC38 model is still ongoing. In these models the Triple was well tolerated with mice showing similar final body-weight changes compared to the other groups including the vehicle-group.

Conclusions: DZB is well-tolerated when combined with paclitaxel and a VEGFR2-Ab in murine syngeneic models, and shows an additive effect in the orthotopic breast models. These data support the ongoing clinical trial with DZB in GC (FIDES-03, NCT04604132).

No conflict of interest.

156 (PB036)

KRASG12C Inhibitor, VRTX126 in combination with Tyrosine Kinase Inhibitor, leads to pronounced and effective response in G12C-mutated cancers

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Background: KRASG12C mutation occurs in about *13% of NSCLC, 4% of colorectal and ~2 % of patients with other solid tumors*. Despite of significant progress made with KRASG12C inhibitors, limited clinical benefit was bestowed in reported data, which is otherwise is expected from these selective agents. T*reatment with a KRASG12C inhibitor does lead to initial oncoprotein signaling inhibition but has been reported to be accompanied with reactivation of alternative MAPK pathway, including bypassing inhibition without actually affecting target inactivation*. Given the multiple factors leading to acquired resistance reported by this class of inhibitors, co-targeting through a combination of *KRASG12C inhibitor with TKIs or other downstream/upstream inhibitors of MAPK pathway is imperative and is expected to provide sustained and more durable response in patients with KRASG12C mutations.*

Methods: VRTX126, was recently reported as a *potent and selective inhibitor of KRASG12C with an IC50 of 0.7 nM (pERK, H358)*. Here, we report *synergistic effect of VRTX126 with Afatinib (*a tyrosine kinase inhibitor*)* in an MTT based cell viability assay, using 96-well plate platform in a dose response matrix. Additionally, in vivo combinability of VRTX126 and Afatinib was ascertained in a Xenograft model of NCI-H358 using Female Balb/c Nude Mice. Protein Western blot analysis and Immunohistochemistry (IHC) for downstream KRAS pathway markers were also determined in cell lysates and tumor samples. Long read sequencing, to understand the mechanistic transcriptomic signature profile and the mechanism of crosstalk of intersecting pathways has also been investigated at both invitro and in vivo settings.

Results: *Dose response matrix* in H358 cell lines indicated *several fold reduction in IC50 of Afatinib in combination with VRTX126*. VRTX126 (15 mg/kg/QD) with Afatinib (12.5 mg/kg/QD) exhibited significant (>80%) tumor growth inhibition (TGI), indicating a *significant reduction in the effective dose of Afatinib when used in combination with VRTX126*, in this model. A pronounced suppression of biomarkers (pERK, pS6 and DUSP6) and IHC analysis indicated complete inhibition of KRAS-mediated pathway demonstrating desired downstream signal modulation.

Conclusions: In vitro study using a combination matrix of VRTX126 and Afatinib indicated synergistic effect leading to a significant dose reduction of TKI which was further confirmed in in-vivo settings. This paves way for *safely combining VRTX126 with multiple precision medicines*, including TKIs at significantly lower doses.

No conflict of interest.

158 (PB038)

RVU120, a small molecule inhibitor of CDK8/19 kinases, enhances rituximab-driven NK cells-mediated cytotoxicity both in vitro and in vivo

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Background: NK cells act as one of the most important immunosurveillance mechanisms eliminating cancer cells. NK cells recognize Fc portions of antibodies binding to surface antigens of cancer cells and release cytotoxic granules and cytokines. Antibody-dependent cell-mediated cytotoxicity (ADCC) is one of the major NK-dependent killing mechanisms, activated for example by therapeutic monoclonal antibodies, such as the anti-CD20 antibody rituximab. NK cell activity is attenuated by STAT1 (S727) phosphorylation, mediated by the CDK8 kinase. Here we show results of combination therapy of anti-CD20 antibody rituximab with a clinical stage CDK8/19 small molecule inhibitor RVU120.

Material and methods: The effect of RVU120 or rituximab as well as their combination on human NK cells was tested in vitro in a co-culture cell killing assay using effector NK cells isolated from healthy donors and a panel of CD20-positive diffuse large B-cell lymphoma (DLBCL) cell lines as target