B08

BLOCKING THE STRESS SPECIFIC APIM-PCNA INTERACTION INCREASES THE EFFICACY OF CANCER THERAPIES

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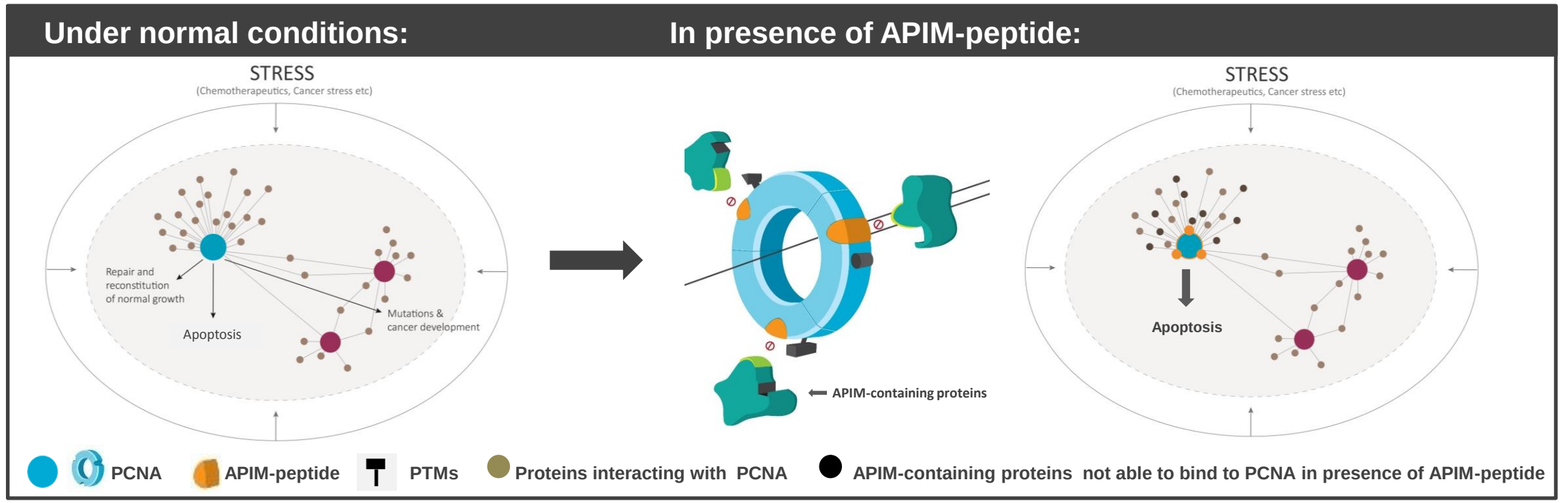


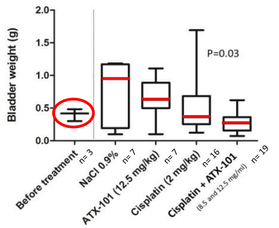
APIM-PEPTIDE IMPAIRS THE DNA DAMAGE RESPONSE (DDR). PCNA has a central role in regulating cellular homeostasis. Posttranslational modifications (PTMs) on PCNA after cellular damage/cellular stress increase the binding affinity of APIM. APIM-peptide therefore selectively blocks the interaction between PCNA and APIM-containing proteins involved in cellular stress mechanisms. APIM-peptide thus impairs the balance between repair and reconstitution, apoptosis and tolerance mechanisms, and leads to **cancer cell hypersensitivity to chemotherapeutics, targeted agents and γ- irradiation.**

The APIM-peptide ATX-101 (MDRWLVK-W-KKKRK-I-RRRRRRRRRRR) is currently being developed for use in cancer therapy by the NTNU spinoff APIM Therapeutics. ATX-101 has a favorable toxicology profile (GLP program completed) and CTA-ready; clinical entry in 2016.

PCNA CONTAINS TWO INTERACTION MOTIFS, PIP-box AND APIM

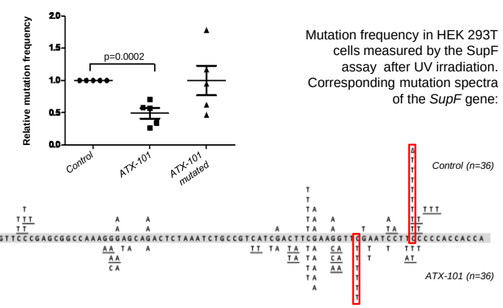
- **>200 proteins predicted to contain APIM;** most of them are involved in DNA damage and cellular stress responses
- **PCNA is essential for DNA metabolism, i.e. replication and repair, mutagenesis/ translesion synthesis (TLS), epigenetics.** Many proteins involved in these processes contain APIM. APIM-peptide reduces DNA repair and TLS (e.g. ZRANB3, Topo IIα, RAD51B, XPA, POL ζ and TFII-I contain APIM) .
- **Has a role in cellular signaling, metabolism, inflammation and immunity** Many proteins involved in cellular signaling, including several kinases contain APIM. APIM-peptide affects major signaling pathways, including PI3K/AKT and MAPK.

The APIM–peptide ATX-101 targets PCNA and increases the efficacy of cisplatin:



(A27 cancer cells in Fisher rat, an immune competent orthotopic syngenic rat muscle invasive bladder cancer model. Treatment: intravenous injections, 1x/week)

ATX-101 inhibits mutagenesis/translesion synthesis (TLS):



Mutation frequency in HEK 293T cells measured by the SupF assay after UV irradiation. Corresponding mutation spectra of the *SupF* gene:

Conclusions:

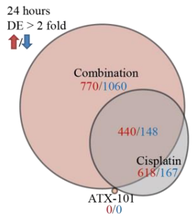
- The APIM-peptide ATX-101 targets PCNA and reduces DNA repair and TLS, and thereby increases the efficacy of chemo- and radiotherapy.
- ATX-101 reduces mutagenesis
- Gene expression analysis reveals a massive increase in DEG when cisplatin is combined with ATX-101. Many of these genes are involved in regulation of apoptosis, metabolism and cellular signaling (EGFR/AKT/MAPK).
- Protein levels of multiple signal molecules involved in DDR, including kinases, phosphatases, ubiquitin ligases are changed after ATX-101+cisplatin treatment.
- Overall small changes in metabolite pools, however specific central metabolites (e.g. ATP, fructose 1,6- biphosphate) are changed.
- Targeting PCNA with ATX-101 increases the anti-proliferative efficacy of kinase inhibitors and changes the kinase activities in harvested tumor tissues.

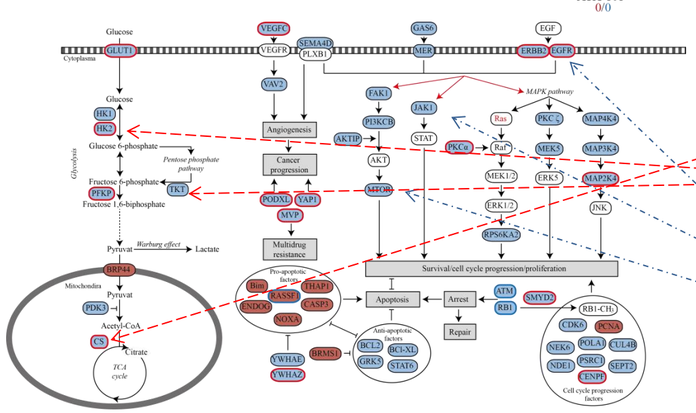
Gene expression analysis, kinome analysis and metabolite profiling of cells treated with an ATX-101+cisplatin show extensive changes compared to single treatments:

- **Many of the differentially expressed genes (DEG) in the combo-treatment are involved in regulation of apoptosis, metabolism and EGFR/VEGFR signaling. Illustrated in figure below (left panel).**
- **Kinome analysis (MS-based pull down assay, MIB) shows correlation with DEG for important kinases and clear effects of ATX-101**
- **Metabolite profiling reveals changes in glycolytic intermediates, amino acids and ATP pools (bottom)**

DEG: Two bladder cell lines (Um-Uc 3 and T24), were treated with ATX-101+ cisplatin (24h), three biological replicas of each. Only genes found in all six experiments are included. ~1200 DEGs FOUND ONLY IN COMBINATION

Illustration below:
Background: genes up/down reg. in our data
Edges: genes commonly found up/down reg. bladder cancer



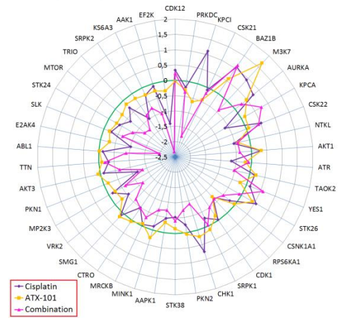


Metabolite profiles: ATX-101 + cisplatin treated Um-Uc 3 and T24 cells (24h) analyses by three LC-MSMS methods reveal:

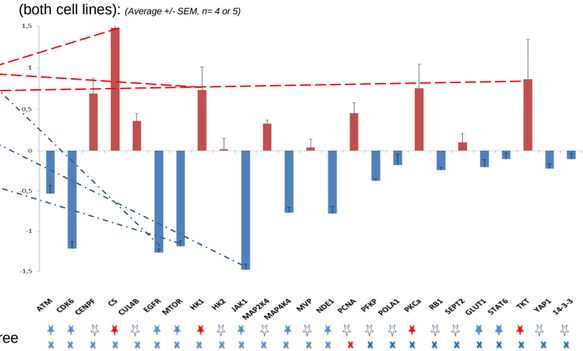
- No changes in energy charge (EC) between ATX-101+ cisplatin combination vs control even though ATP pool is **down** in Um-Uc 3 (EC > 0.9 for all conditions)
- DHAP/G3P and fructose 1,6- biphosphate **up.** Increased pull down of **HK1** and **TKT** (proteome/kinome)
- Hexose-phosphates, 3PG, 2PG and PEP no changes
- Small changes in amino acid pools in T24, however Ala and Ile >2x **down** in Um-Uc 3
- Lactate in T24 **up**, no change in Um-Uc 3

Kinome analysis: Treatment of Um-Uc 3 and T24 with ATX-101+ cisplatin (24h) leads To massive changes in kinases, phosphatases, ubiquitin ligases and other proteins involved in cellular signaling compared to cisplatin and ATX-101 only.

Changes in pull downs relative to untreated control *(T24, log2) :*

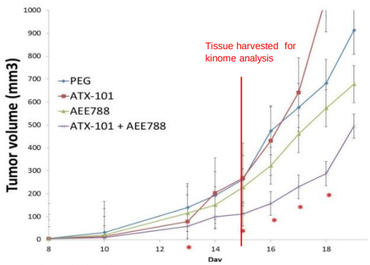


Histogram: changes (log2) in pull down from ATX-101 + cisplatin treated relative to untreated control with focus on DEG genes frequently dysregulated in bladder cancer (both cell lines): *(Average +/- SEM, n= 4 or 5)*



Key references: *Müller, R. et al. PloS one 8, (2013). Olaisen C, et al. Cellular signalling 27, (2015). Gilljam, K. M. et al. J Cell Biol 186, (2009). Gilljam, K. M., PloS one 7, (2012). Gederaas, O. A. et al.. Transl. Oncol 7, (2014), Ciccia et al. Mol Cell 43, (2012).*

ATX-101 increases the efficacy of AEE788, a HER1(EGFR)/2, VEGFR1/2 inhibitor, in an orthotopic syngenic breast cancer mouse model:



(67NR/BalBC, immune competent. Treatment: 3x/week. PEG/DMSO, PO, n=7. ATX-101 6 mg/kg, IP, n=7. AEE788 25 mg/kg, PO, n=12. ATX-101 6 mg/kg + AEE788 25 mg/kg, IP/PO, n=12.)

Kinome analysis of tumor tissues shows that ATX-101 affects the kinase activities *in situ*:

Different kinases are active in tumor extracts from ATX-101+ AEE788 treated animals compared to AEE788 and ATX-101 single treated animals (PamGene, Ser/Thr chip assay)(n=6)

Upstream kinase analysis: >0 activity reduced relative to control, <0 activity increased relative to control (log2)