P2.1

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

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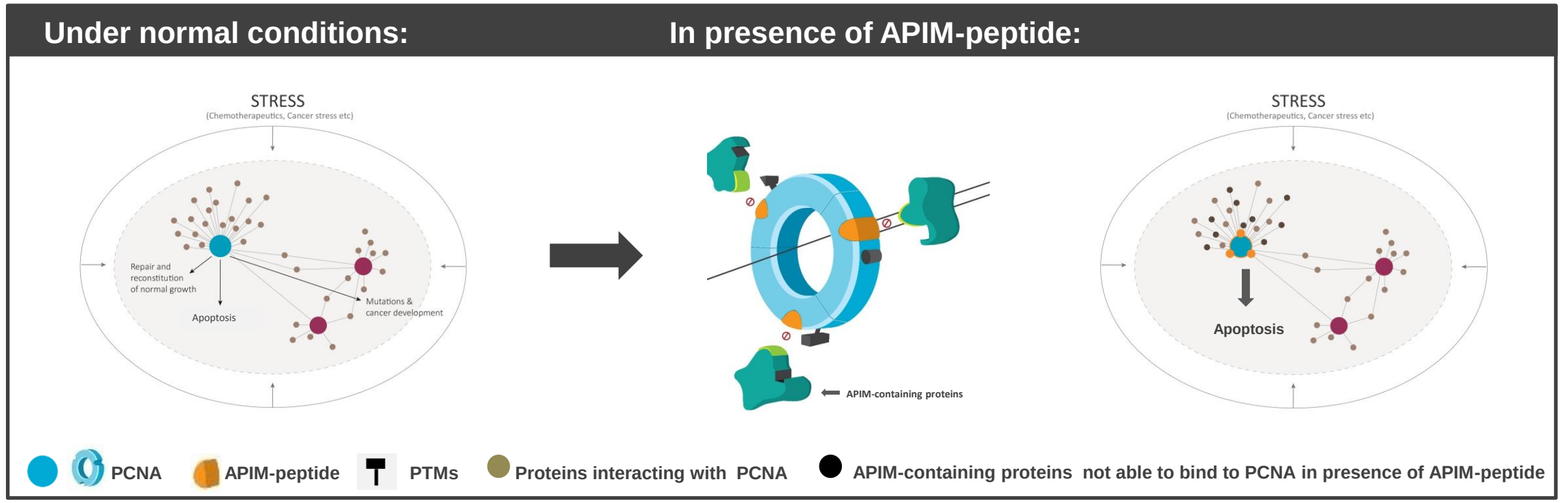
APIM Therapeutics
Improving Chemotherapy for Cancer Patients

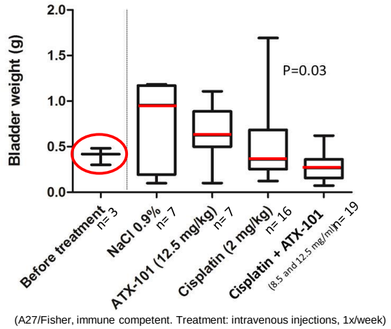


APIM-PEPTIDE IMPAIR THE CELLULAR STRESS DEFENSE MECHANISMS. PCNA has a central role in regulating cellular homeostasis. Posttranslational modifications (PTMs) on PCNA after cellular damage/cellular stress increase the binding affinity of APIM. APIM-peptide therefore selectively blocks the interaction between PCNA and APIM-containing proteins involved in cellular defense mechanisms. APIM-peptide thus impair the balance between repair and reconstitution, apoptosis and tolerance mechanisms, and leads to cancer cell hypersensitivity for chemotherapeutics, targeted agents and γ-irradiation.
The APIM-peptide ATX-101 (MDRLVK-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 CONTAIN TWO INTERACTION MOTIFS, PIP-box AND APIM

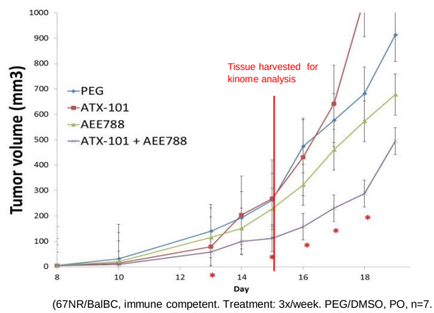
- **>200 proteins predicted to contain APIM;** most of them are involved in DNA damage and cellular stress responses
- **PCNA is essential for DNA metabolism, i.e. replication and repair, mutagenesis/ translesion synthesis (TLS), epigenetics.** Many proteins involved in DNA repair, TLS and epigenetics contain APIM. APIM-peptide reduce DNA repair and TLS.
- **Have 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 increase the efficacy of cisplatin in an orthotopic syngenic rat muscle invasive bladder cancer model:



(A27/Fisher, immune competent. Treatment: intravenous injections, 1x/week)

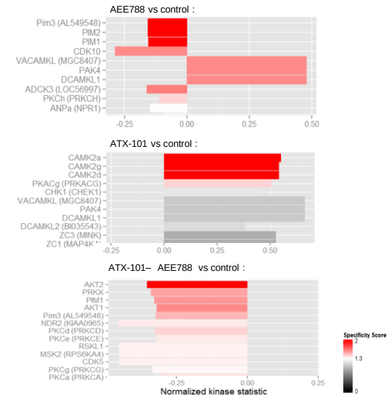
ATX-101 increase the efficacy of AEE788, a HER1/2, EGFR, VEGFR1/2 inhibitor, in an orthotopic syngenic breast cancer mice model:



(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: 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



Conclusions:

- The APIM-peptide ATX-101 targets PCNA and increases the efficacy of chemotherapeutics and targeted anti-cancer drugs.
- ATX-101 affects kinase activities in tumor tissues.
- Gene expression analysis reveals a massive increase in DEG when cisplatin is combined with ATX-101. These genes are involved in regulation of apoptosis, metabolism and cellular signaling (EGFR/AKT/MAPK). ATX-101 alone leads to no DEG.
- Protein levels of hundreds of kinases, phosphatases, ubiquitin ligases and other signaling molecules are changed after ATX-101–cisplatin treatment.
- Good correlation between DEG and changes found in the kinome after ATX-101–cisplatin treatment.
- Majority of metabolite pools unchanged, while specific central metabolites (e.g. ATP, fructose 1,6- biphosphate) changed.

Gene expression analysis, kinome analysis and metabolite profiling of cells treated with an ATX-101-cisplatin combination 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) correlates well with data from gene expression analysis (right panel)
- 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

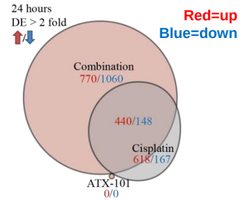
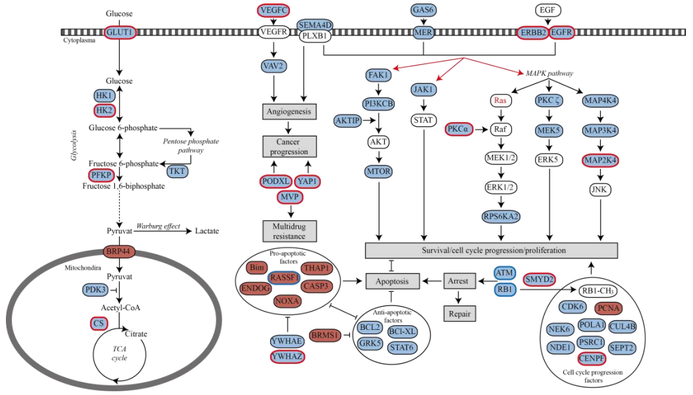


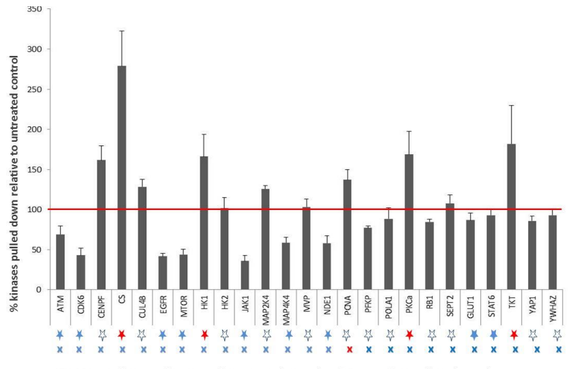
Illustration below:
Background: genes **up**/**down** reg. in our data
Edges: genes commonly found **up**/**down** reg. bladder cancer



Metabolite profiles: ATX-101- cisplatin treated of 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**
- 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 several hundred kinases, phosphatases, ubiquitin ligases and other proteins involved cellular signaling in bladder cancer cells. Changes relative to untreated control found in both cell lines (average +/- SEM, n= 4 or 5) shown below (only a fraction of total proteins changed). Compared with changes for cisplatin only and DEG.



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).*