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

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

Motion frequency in HDX-20T cells measured by the Sopp assay, 48h UV irradiation. Corresponding infection rates of the Sopp assay for each conditions.

Conclusions:
- The APIM peptide ATX-101 targets PCNA and reduces DNA repair and TLS, and thereby increases the efficacy of chemotherapeutic agents.
- 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-bisphosphate) 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, kinase 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.
- Changes in pull downs: changes (log2) in pull down from ATX-101, cisplatin and control (log2).

**Metabolite profiles:** ATX-101 + cisplatin-treated UuM-uc 3 and T24 cells (24h) analysed by three LC-MS/MS methods revealed:
- No changes in energy charge (EC) between ATX-101 + cisplatin combination vs control even though ATU pool is down in UuM-uc 3 (EC > 0.9 for all conditions)
- DNA/GTP and fructose 1,6-bisphosphate up; Increased pull down of HK1 and TTK (metastasis marker)
- Hes-1 decreases, 3PG, 2PG and PEP no changes
- Small changes in amino acid pools in T24, however AAs and tRNA down in UuM-uc 3
- Lactate in T24 up, no change in UuM-uc 3

**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 (MDRWHK-W-KKKIK-LRRRRRRRRRRRR) is currently being developed for use in cancer therapy by the NTNU-sponsored APIM Therapeutics. ATX-101 has a toxicology profile (GLP program completed) and CTA-ready; clinical entry in 2016.

**Under normal conditions:**
- PCNA-containing proteins
- APIM-peptide
- PTMs
- Proteins interacting with PCNA

**In presence of APIM-peptide:**
- APIM-containing proteins not able to bind to PCNA in presence of APIM-peptide

**PCNA CONTAINS TWO INTERACTION MOTIFS, HP-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, Trao U1, RAD51B, XPA, POL 1 and TFIIB containing APIM).
- Has a role in cellular signalling, metabolism, inflammation and immunity
- Many proteins involved in cellular signalling, including several kinases contain APIM. APIM-peptide affects major signalling pathways, including PI3K/AKT and MAPK.

**ATX-101 increases the efficacy of AEE788, a HER1/EGFR2, VEGFR1/2 inhibitor, in an orthotopic syngeneic breast cancer mouse model:**

**Kinase analysis of tumor tissues shows that ATX-101 affects the kinase activities in all Tissues:** Different kinases are active in tumor extracts from ATX-101 + AEE788-treated animals compared to AEE788 and ATX-101 single treated animals. (PamGene, SarTHR chip assay (n=6))

Upstream kinase analysis: α) activity reduced relative to control, β) activity increased relative to control (log2)