V36.5
Improving oncolytic virotherapy efficacy using target therapies against molecules of cell cycle control

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Objective: Successful and effective replication is a key requirement in oncolytic virotherapy that has to be improved. We investigated the molecular mechanism of cell cycle inhibitors in greater detail to develop an improved therapy design to enhance efficacy of the oncolytic adenovirus XVir-N-31 in bladder cancer cells.
Methods: Bladder cancer cell lines (UMUC3, T24, RT112, 253J, 647V, 639V) were infected/treated with XVir-N-31 and/or small molecule inhibitors against Checkpoint kinase 1 (Chk1) (UCN-01, AZD7762) or CDK4/6 (PD-0332991, LEE011, LY-2835219). Cell viability was assessed using SRB assay and biochemical effects were examined by immunoblotting. Virus replication and titer was determined by using qPCR and a titer test on Hek293 cells. siRNAs were used to interfere with CHK-1, E2F1-3 and RB expression level.
Results: Combining viral titer that do not result in cell death with targeted therapies revealed that specific targeting of CDK4/6 induced cell death of 70–90% compared to monotherapy with the inhibitors used. Rb negative cell lines, resistant to PD-0332991, did not show additional effects on cell viability upon combination treatment. Previously described effects of UCN-01 are due to its ability to also target CDK4/6 but specific CHK-1 inhibition had no effects on virus induced cell death and replication. An increase of 5–10 fold was observed upon use of CDK4/6 inhibitors, an effect that was partially mediated by E2F1. Also, particle formation improved 3–5 fold.
Conclusion: Oncolytic adenovirotherapy can be improved in combination with specific CDK4/6 inhibitors in Rb+ bladder cancer cell lines. This suggests a novel molecular mechanism for viral replication that contradicts the current model in which E2F1 is a positive regulator for replication.

V36.6
Cell cycle arrest in G1-phase by Cdk4/6 inhibitors dramatically enhances replication of adenoviruses via inhibition of the Rb/E2f pathway

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Retinoblastoma proteins (Rb) are key regulators of exit from the G1-phase of the cell cycle. Hyperphosphorylation of Rb inhibits its binding to E2F and leads to stimulation of cell proliferation. CDK4/6 inhibitors such as Palbociclib suppress the proliferation of Rb positive tumors and inducing G1-arrest by down-regulation/degradation of RB, pRb and E2F.
Adenovirus normally infects non-cycling cells, which are poor hosts for viral replication. Consequently, these viruses have evolved proteins such as E1A that force the host cell into the cell cycle and induce the expression of the cellular biosynthetic machinery and substrates that are required for efficient production of viral progeny. In summary, E2F is released from Rb post adenovirus infection by E1A and thereby driving virus replication and cell cycle progression. Thus, on this basis a combination of CDK4/6 inhibitors and adenoviruses seems not be appropriate and should rather work antagonistic than synergistic.
However, here we show that adenovirus replication, particle formation and cell killing is dramatically enhanced by CDK4/6 inhibitors. Analyzing the underlying mechanism for this surprising effect using siRNA against E2F and a specific adenovirus mutant with a deletion in the E2F-bindings sites in the E2-early promoter we discovered, that E2F-1 does not possesses an activating function, but instead suppresses adenovirus E2-promoter activity, which proteins including viral polymerase are essential for viral replication.
In light of our results we will present a revised model of the role of E2F in the adenovirus life cycle and present data in conjunction with YB-1 based virotherapy.

V36.7
Characterisation of bladder cancer drug responses in organoids and *ex vivo* models

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Objective: Bladder cancer (BLCa) is the fourth common cancer in European men. Non-muscle invasive BLCa (NMIBC) and muscle invasive BLCa (MIBC) have specific clinical profiles and require different treatment approaches. However, they underrepresent the clinical complexity of patients. Thus, characterisation of BLCa subtypes is crucial to account for tumor heterogeneity. Lately, effectiveness of cisplatin therapy was linked to four molecular subtypes of MIBC. Pre-clinical models, such as patient-derived organoids (PDOs) and *ex vivo* tissue culture, are useful tools to screen drugs and tailor medical care to a patient's individual genetic background. Here we aim to characterise drug responses of BLCa samples and correlate to molecular subtypes based on transcriptomic profiles.
Methods: Human NMIBC and MIBC samples were treated *ex vivo* with cisplatin and gemcitabine. Drug response and tumor subtype were studied by immunohistochemistry and sequencing. Transcriptomics of PDOs was analysed and drug responsiveness evaluated by viability assay.
Results: The phenotype of parental tumor was maintained *ex vivo* and different samples responded differentially to the tested drugs. Similarly, drug screens on PDOs revealed a pattern, specific to a particular tumor subtype. Basal subtypes responded most favourably to cisplatin and cisplatin/gemcitabine combination treatment, whereas a luminal NMIBC sample was insensitive to cisplatin. Sequencing of *ex vivo* treated tissue and PDOs revealed distinct molecular profiles.
Conclusions: Patient-derived BLCa responds differentially to drug treatments. Future studies will show if our pre-clinical findings correlate to clinical and molecular parameters to improve patient stratification and treatment regimens.

V36.8
The role of interleukin-1-receptor-antagonist in bladder cancer migration and invasion

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The interleukin-1-receptor antagonist IL1Ra (encoded by the IL1RN gene) is a potent competitive antagonist to Interleukin-1 (IL-1) and thereby mainly involved in regulation of inflammation. Previous data found a loss of IL1RN expression in muscle-invasive urothelial carcinoma of the bladder (UCB) as well as an IL-1 dependent decrease in vascular endothelial barrier function. Here we investigated the potential role of IL1Ra in cancer cell invasion in vitro.
IL1RN was ectopically expressed in the invasive human UCB cell line T24. Cell migration and invasion were continuously monitored using the xCELLigence RTCA DP device. It features a Boyden chamber-like set-up with an upper and lower chamber separated by a microporous membrane with the bottom chamber containing a chemoattractant. In some experiments the bottom of the upper chamber was additionally coated with Matrigel, simulating the basement membrane matrix. As cells migrate/invade and adhere to microelectrodes on the underside of the membrane they cause an increase in electrical impedance which was continuously monitored for 48 h. The real-time observation data showed a significant decrease of cell migration and invasion in T24 cells overexpressing IL1RN, compared to the cells