post transcriptional gene silencing (PTGS) or transcriptional gene silencing (TGS). PTGS usually occurs in the cytoplasm and leads to sequence specific degradation of the target mRNA. TGS is a nuclear phenomenon and requires a promoter associated RNA to cause silencing. High risk Human papillomaviruses (HPV 16 and 18) are implicated in pathogenesis of cervical cancer and the current vaccines are only prophylactic and ineffective in the already infected individuals. The oncogenes E6 and E7 are responsible for malignant transformation and ineffective in the already infected individuals. The oncogenes E6 and E7 are responsible for malignant transformation and ineffective in the already infected individuals. The oncogenes E6 and E7 are responsible for malignant transformation and ineffective in the already infected individuals.

Material and methods HPV 18 positive cervical cancer cell line HeLa was treated with an siRNA against E6 mRNA. Its efficacy was assessed by qPCR for the fold expression of E6 and E7. PTGS was shown by a modified protocol of rapid amplification of cDNA ends (RACE) in which siRNA transfection followed by sequence analysis of the amplified product was done to identify the 5’end of the cleaved E6 mRNA. TGS effect was studied using nuclear run-on assay.

Results and discussions There was a significant downregulation of E6 and E7 in cells transfected with E6 siRNA as compared to control siRNA. Sequence analysis of the amplified RACE product confirmed PTGS of the E6 mRNA. Nuclear run on assay revealed a considerable decrease in the levels of freshly synthesised E6 and E7 transcripts suggesting a decreased rate of their transcription.

Conclusion Our study shows single siRNA mediated gene silencing by both PTGS and TGS. PTGS effect is due to perfect complementarity with the target mRNA while its proximity to the transcription start site mediates TGS. In HPV associated cancers, RNAi based therapies have utilised PTGS or TGS activity alone. We show the effectiveness of a single siRNA in mediating both the effects. This approach is more promising than either of the techniques alone and is an effective alternative for other therapies with associated side effects.

Introduction Immunotherapy using monoclonal antibodies (mAbs) or domain resurfaced antibody fragments specifically directed against tumour antigens aids in the destruction of cancer cells. Epidermal growth factor receptor variant III (EGFRvIII), is an extracellular deletion mutant of EGFR with a tumour specific epitope expressed in glioblastomas. The constitutive kinase activity of EGFRvIII leads to inhibitor resistance but responds to immunotherapeutic mAbs. The potential of mAbs are curtailed due to immunogenicity and poor tumour penetration wherein antibody fragments work better. The current study focuses on the generation of humanised antibody variable fragments in baculovirus expression system using anti-EGFRvIII murine antibody template.

Material and methods The antibody variable fragment constructs were designed by bioinformatic tools (Modeller) and the best models were selected using ERRAT, Ramachandran and root-mean-square deviation (RMSD) values. The anti-EGFRvIII antibody fragment was cloned into baculovirus acceptor vector and the resurfacing or humanising mutations were introduced in the variable regions via site-directed mutagenesis and verified by sanger sequencing. Recombinant viruses were generated using MultiBac expression system. Protein purification was done using His-tag based affinity
chromatography and verified on a western blot. Immunocytochemistry and immunohistochemistry were carried out to confirm binding efficiency of these constructs to EGFRvIII expressing cell lines and glioblastoma sections respectively.

**Results and discussions** In this study, five domain resurfaced anti-EGFRvIII constructs were selected by bioinformatic analysis. The humanising mutations were introduced in the variable fragments via site-directed mutagenesis and confirmed from the sequencing chromatogram. The recombinant proteins were expressed and the purified protein was detected at 25 kDa on a western blot. Immunocytochemistry and immunohistochemistry studies with the antibody variable fragments demonstrated intense binding or brown staining of EGFRvIII expressing cell lines and glioblastoma patient sample sections.

**Conclusion** Hence, we have generated recombinantly engineered anti-EGFRvIII constructs using baculovirus expression system. The targeting ability of the constructs was studied *in vitro* and in glioblastoma patient samples. These antibody variable fragments can be used as effective immunotherapeutic agents against EGFRvIII mutant expressing cancers.

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**PO-008** ANTI-TUMOUR PROPERTIES OF NOVEL MULTIKINASE INHIBITORS IN SARCOMAS: SYNERGISTIC COMBINATION WITH DOXORUBICIN

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**Introduction** Cytotoxic drugs like doxorubicin remain as the most utilised agents in sarcoma treatment. However, advanced sarcomas often show resistance to these drugs, mainly through the overexpression of members of the ATP binding cassette (ABC) family which act as efflux pumps for drugs. Therefore, the development more effective treatments able to prevent drug resistance would improve sarcoma treatments.

Multi-kinase inhibitors provided an efficient way to target several pro-tumorigenic pathways using a single agent and have shown anti-tumour activity in a range of tumours. Abarant activation of pro-tumoral kinase is common in sarcoma, however the effect of multikinase inhibitors in sarcoma has been barely tested. Here, we aimed to study the anti-tumour effect of one of such inhibitors in cell-of-origin sarcoma models and sarcoma patient-derived primary cell lines, as well as its ability to prevent drug resistance and synergize with doxorubicin.

**Material and methods** Cell survival, apoptotic induction, cell cycle progression and DNA damage were analysed after drug treatments. The existence of synergism between drugs was evaluated using statistic tools. Drug effect on signalling proteins was studied using phospho-antibody arrays and Western blotting analysis. Interaction between drugs and ABC transporters were characterised using substrate and inhibition assays. Finally, *in vivo* tumour growth and pharmacodynamic response after drug treatments were evaluated in xenografts models.

**Results and discussions** Sarcoma cells were sensitive to submicromolar concentrations of the multikinase inhibitor, which induced cell cycle arrest, DNA damage and apoptosis. Evaluation of the phosphorylation status of signalling kinases evidenced that PI3K/AKT/mTOR and ERK1/2 were the most highly activated pathways in sarcoma cells and that the drug efficiently inhibited them *in vitro* and *in vivo*. By using specific mTOR inhibitors and agonists, we confirmed that the inhibition of this pathway contributed to the cytotoxic effect of the drug. In addition, this drug inhibited the expression and activity of ABC transporters and was not a substrate for them. In line with this ability, we found a synergistic cytotoxic effect when sarcoma cells were treated with combinations of the kinase inhibitor and doxorubicin both *in vitro* and *in vivo*.

**Conclusion** A novel multikinase inhibitor induced a consistent cytotoxic effect and was able to counteract drug resistance in sarcoma cells, thus highlighting its therapeutic potential when combined with current treatments.

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**PO-009** A NOVEL INTEGRIN ALPHA 5 BINDING PEPTIDE POTENTIATES EFFECTS OF CHEMOTHERAPY IN PANCREATIC CANCER

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**Introduction** Cancer-associated fibroblasts (CAFs) are the key cell type in the pancreatic tumour microenvironment, which induces tumour growth and metastasis. The extracellular matrix produced by CAFs also acts as a barrier to chemotherapy. We have recently identified integrin alpha5 as a novel target overexpressed in CAFs. In this study, we have designed a novel integrin binding peptide (so-called AV3) against ITGA5, comprised of 7 amino acids, and studied its significance in pancreatic cancer.

**Material and methods** AV3 and AV3-PEG-5FAM (AV3-FAM) peptides were custom synthesised. Microscale thermophoresis (MST) was performed to determine the binding affinity of the fluorescently labelled peptide (AV3-FAM) against α5β1 and α4β1 receptor. AV3-FAM binding on primary human pancreatic stellate cells (hPSCs) was studied either with fluorescent microscopy or flow cytometry. Using qPCR and western blot analyses, AV3 therapeutic efficacy was studied on hPSCs. *In vivo* studies were performed in (Panc-1 + hPSCs) co-injection tumour model and patient-derived xenograft (PDX) tumour to assess the therapeutic value of AV3.

**Results and discussions** AV3-FAM showed a specific and high binding affinity (Kd 0.97 nM) to α5β1 but not to a close family integrin α4β1, indicating its specific binding to α5 (ITGA5). *In vitro*, activation of hPSCs with recombinant TGFβ1 significantly induced ITGA5 expression. AV3-FAM showed a strong binding to TGF-β-activated hPSCs was confirmed using flow cytometric analysis and fluorescent microscopy. Furthermore, we examined whether AV3 is able to block ITGA5 and thereby inhibit hPSCs activation. Interestingly, treatment with AV3 led to inhibition of TGF-β-induced differentiation of hPSCs, as shown with qPCR and western blot analyses. In addition, AV3 also inhibited TGFβ- induced contractility and p-FAK signalling in hPSCs. *In vivo*, treatment with AV3 (either