Introduction
Overlay survival (OS) of patients with pancreatic ductal adenocarcinoma (PDAC) is extremely poor. Of patients eligible for surgery (20%), around 15% present with a recurrence within 6 months (m), while 10% survive over 5 years after diagnosis. Detailed clinicopathological and molecular knowledge of factors influencing survival may lead to better prognostic and/or predictive factors and preselection of individual patients for specific treatment strategies.

Material and methods
Fresh frozen PDAC resection specimens from the Academic Medical Centre Amsterdam (1993–2015) were histopathologically revised, and clinicopathological details were collected. From samples with a tumour cellularity of ≥30% (n=90), mRNA, miRNA, and DNA were used for NGS at multiple levels. Corresponding FFPE blocks were selected for tissue microarrays.

Results and discussions
Unsupervised consensus clustering of gene expression profiles of 90 PDAC revealed four subgroups with divergent OS rates: secretory (14.7 m), epithelial (31.8 m), compound pancreatic (21.5 m), and mesenchymal (14.0 m) subgroups (p=0.002). The differences between the subgroups suggest that more targeted therapy may be feasible for PDAC.

The epithelial and mesenchymal subgroups show an upregulation of genes related to DNA repair and hypoxia, respectively, suggesting sensitivity to hyperthermia, which is known to enhance cytotoxic radio-, and chemotherapy effects through frustration of tumour DNA repair and/or better oxygenation of hypoxic tumours. Immune-related gene expression was observed to be upregulated in the compound pancreatic and mesenchymal subtypes; differences were seen in expression of PDL-1, type I interferon, and MHC Class I, which may explain the difference in OS and suggests differential responses to immunotherapeutic agents. Finally, the epithelial subtype is characterised by prominent increases in metabolic signatures. Differences in metabolic flexibility suggest differences in response to mTOR inhibitors.

To determine clinical relevance and identify biomarkers, we are currently characterising the subtypes at the miRNA, mutational, copy number, and immunohistochemical level.

Conclusion
In our well-defined single-centre set of 90 PDAC, we identified four transcriptomics-based subgroups with different survival outcomes that show a correlation with altered DNA-repair-, hypoxia, immune-, and metabolic features. Complete characterisation of these liabilities may help to further identify prognosticators, and preselect patients for specific treatment strategies.
may improve drug screening in-vitro to identify improved chemotherapy. The CRIS classifier identifies five CRC intrinsic subtypes within the cancer cell transcriptome: A, B, C, D and E. This classifier improves cell line subtype mapping by considering only tumour cell transcripts. The inhibitor of apoptosis (IAP) proteins are promising targets in personalised medicine as CRC tumours dysregulate IAPs to avoid apoptosis. This study explored the efficacy of Birinapant - a potent SMAC mimetic and antagonist of IAPs, along with synergistic agents to promote optimal cell death in vitro using a model of CRC intrinsic subtypes.

Material and methods The CRIS classifier analysis was performed on 150 CRC cell transcriptomes to select 10 cell lines: CRIS A (DLD-1, LS174T), CRIS B (LoVo, HT29), CRIS C (Gp5D, LIM1215), CRIS D (HCT116, RKO) and CRIS E (HCT116 p53−/−, LS513). The cells were treated with 1 μM Birinapant alone, and/or the cytotoxic pair Oxaliplatin and 5-Fluorouracil (2 μM, 10 μM), and/or TNF-alpha. Post-treatment cellular sensitivity was measured at 24, 48 and 72 hours by assessing Annexin V/Propidium Iodide positivity using high content microscopy.

Results and discussions The cell death data from the entire panel showed that Birinapant cannot induce apoptosis when administered alone. However, Birinapant elicits synergistic effects in combination with Oxaliplatin/5-Flourouracil, or the pro-inflammatory cytokine TNF-alpha. The greatest degree of sensitivity was observed in the HCT116 and RKO CRIS D cell lines and least in the DLD1 and HT29 CRIS A subtypes. Of note, the p53-deficient HCT116 CRIS E model was less sensitive to the combined effects of Birinapant and chemotherapy, suggesting an important role for p53 in mediating this synergy.

Conclusion Future work will expand the analysis to more CRIS D cell lines and investigate the molecular mechanisms behind the p53-dependence of the chemotherapy/IAP antagonist interactions. These results have clinical relevance as in parallel studies, we have shown that patients with CRIS D subtype tumours have a suboptimal response to standard-of-care chemotherapy.

REFERENCE
1. WL Allen et al., JCO Precision Oncology, 2018 (in press)

Monday Poster Presentation

Experimental/Molecular Therapeutics, Pharmacogenomics

New Therapies

THERAPEUTIC INHIBITION OF TREFOIL FACTOR 3 (TFF3) DECREASE ONCOGENIC BEHAVIOUR IN NON-SMALL CELL LUNG CANCER (NSCLC)

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Introduction Increasing evidence has shown that trefoil factor 3 (TFF3) is increased in expression in various cancers and promotes cancer progression. Elevated TFF3 levels have been reported in lung cancer patients. TFF3, which is expressed in the vast majority of lung adenocarcinomas (ADC), has also been proposed as a novel biomarker to distinguish between lung ADC and squamous-cell carcinoma (SCC). Herein, we investigated the oncogenic functions of TFF3 and explored the potential of therapeutic TFF3 inhibition in NSCLC.

Material and methods The function of TFF3 in lung ADC was investigated by either forced or depleted expression of TFF3 in H1299 and H1975 cell lines. Cell functional assays performed include total cell count, PI-Annexin V apoptosis assay, cell cycle analysis, soft agar colony formation, 3D growth in Matrigel, wound healing and transwell migration and invasion assays. A novel small molecule TFF3 inhibitor, AMPC, has been developed in and was used to examine the functional effects of TFF3 inhibition in the lung ADC cells, both as a single agent and in combination with MEK1/2 inhibitors. The paracrine effect of local TFF3 production by lung ADC cells on non-TFF3 expressing lung SCC cells was examined using co-culture assays.

Results and discussions The forced expression of TFF3 enhanced cell proliferation and survival, increased anchorage-independent growth, and promoted cell migration and invasion in lung ADC cells. In contrast, the siRNA-mediated depletion of TFF3 expression decreased the oncogenicity of these cells. Consistently, the inhibition of TFF3 by AMPC resulted in markedly decreased cell survival, proliferation, 3D growth and foci formation of lung ADC cells. Expression of the proto-oncogene ARAF was increased by forced expression of TFF3 in lung ADC cells, with consequent increased activation of downstream MEK1/2 and ERK1/2. This TFF3-mediated activation of the MAPK/ERK pathway was required for TFF3-stimulated 3D-growth of lung ADC cells. Moreover, the combination of MEK1/2 inhibitors with AMPC exhibited synergistic inhibitory effects in lung ADC cells. In addition, we demonstrated that the local secretion of TFF3 from lung ADC cells exerts paracrine effects on lung SCC cells, which is relevant in the adenosquamous carcinoma (ASC) subtype of NSCLC.

Conclusion TFF3 is a crucial oncogene in NSCLC progression. The therapeutic inhibition of TFF3 alone or in combination with conventional MEK1/2 inhibitors are potential strategies in the treatment of NSCLC.

REFERENCE
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SINGLE SRNA MEDIATED POST TRANSCRIPTIONAL AND TRANSCRIPTIONAL GENE SILENCING OF HPV18 ONCOGENES

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Introduction Since its advent RNA interference (RNAi) has found multitude of applications in gene silencing and cancer therapeutics. Double stranded RNAs (dsRNAs) specifically and effectively downregulate the target gene and subject to the design of the targeting dsRNA, RNAi operates in the form of