(EC_{50} > 1000 nM). The mutation status of *p53* or *KRAS* did not correlate with the potency of TAK-960 in the cell lines tested in this study. In addition, EC_{50} values in MDR1-overexpressing cell lines were similar to those in cell lines that do not express MDR1.
Conclusions: TAK-960 is a potent, selective PLK1 inhibitor with broad range proliferation inhibition activities including MDR1-expressing tumors. TAK-960 is currently being investigated in phase I clinical trials.

504 POSTER

The role of interferon-gamma- and TNF-induced cell cycle arrest in insulinoma

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Even though most established tumor immunotherapies are based on tumor cell destruction by cytotoxic cells, an increasing number of data shows that successful cancer immunotherapy depends on interferon γ (IFNγ)-producing T cells, i.e. T helper 1 (Th1) cells. RIP1Tag2 mice, where the tumor promoter T-antigen (Tag2) is specifically expressed in β cells, develop well-characterized carcinomas of the pancreatic islets that follow well described multistage carcinogenesis. In a previous study, Tag-specific Th1 cells doubled the lifespan of RIP1Tag2 mice by decreasing the proliferation rate of tumor cells and by inhibiting tumor angiogenesis without causing either tissue destruction or apoptosis in vivo. The therapeutic effect of the Tag-specificTh1 cells was critically dependent on IFNγ and TNF signalling. To unravel the underlying mechanisms, we investigated the direct effects of IFNγ and TNF on malignant β cells from RIP1Tag2 mice and measured in vitro proliferation by the BrdU-proliferation assay and Ki67, analysed the cell cycle progression by flow cytometry and PCR arrays concerning cell cycle genes, and determined the apoptosis rate by TUNEL staining and subG1 analysis. To specify the signalling pathways, we further examined insulinoma from RIP1Tag2xTNFR1$^{-/-}$ (TNF-pathway) and RIP1Tag2xSTAT1$^{-/-}$ (IFNγ-pathway) using the same assays as described above.
We found a significant suppression of the proliferation rate of the isolated RIP1Tag2 tumor cells *in vitro* by IFNγ and TNF that was accompanied by a decrease of the cells in the G2 phase. On the other hand, IFNγ and TNF didn't cause apoptosis (no increase of subG1 cells and negative TUNEL staining). The effects of both cytokines were specific: IFNγ did not block the proliferation of RIP1Tag2xSTAT1$^{-/-}$ cancer cells, and TNF did not block the proliferation of RIP1Tag2xTNFR1$^{-/-}$ tumor cells. Using PCR arrays we found that IFNγ strongly affects the expression of specific cell cycle regulating genes.
Taken together, our data suggest that Tag-Th1-mediated immunotherapy is based on IFNγ- and TNF-dependent repression of insulinoma proliferation by inducing cell cycle arrest in the absence of cell destruction.

505 POSTER

Dual Cdc7/Cdk9 kinase inhibitor, PHA-767491, targets both quiescent and proliferating CLL cells

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Proliferation rate has been recognized as an important factor in the outcome of patients with chronic lymphocytic leukemia (CLL). Proliferation centers, containing dividing CLL cells can be identified in lymph nodes. In this report we show that proliferating CLL cells express active Cdc7 kinase, an S-phase specific kinase essential for DNA replication. Since specific knockdown of Cdc7 induces apoptosis in cancer cells independent of TP53, we decided to evaluate the potential of Cdc7 inhibition in CLL. PHA-767491 is a first in class, prototype Cdc7 inhibitor, which also has cyclin dependent kinase 9 (Cdk9) inhibitory activity.
In this study we assess the activity of PHA-767491 against both quiescent and cells that have been prompted into the proliferative programme using a cellular co-culture system that leads to CD40 stimulation and that mimics lymph node microenvironment.
We find that PHA-767491 is highly active as a single agent in CLL cells purified from peripheral blood of patients regardless of recognized risk factors including TP53 inactivation. PHA-767491 activates Bax leading to mitochondrial dependent apoptosis by decreasing the levels of Mcl-1 at the transcriptional level through inhibition of Cdk9.
We also find that PHA-767491 inhibits replication in proliferating CLL cells following stimulation by CD154 and interleukin-4 (IL-4), with clear evidence of Cdc7 inhibition.
These data show that dual Cdc7/Cdk9 inhibition has the potential to target quiescent and actively proliferating CLL cells and may be a new therapeutic strategy in CLL.

Radiation interactive agents

506 POSTER

Darinaparsin (ZIO-101) is a novel cytotoxic and radiosensitizing agent for prostate cancer

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Background: Darinaparsin (DAR) is a novel organic arsenical (dimethylated arsenic linked to glutathione) with promising anticancer activity. Unlike other arsenicals, DAR appears to have broad spectrum activity in hematologic and solid tumors. Given that DAR appears to have multiple mechanisms of action, including generation of reactive oxygen species (ROS) and arrest of cells in G2/M, we hypothesized that DAR would have significant radiosensitizing effects and efficacy against prostate cancer under both normoxic (NO) and hypoxic (HO) conditions.
Materials and Methods: Experiments were performed in the hormone-independent (HI) and radio-resistant prostate cancer cell line LAPC-4. Cells were treated with DAR at concentrations ranging from 0.01 to 10 uM under either NO or HO (0.5% O2) conditions and irradiated with doses of 0−5 Gy. Viability, proliferation and colony formation were assessed. Mechanistic studies were performed to assess the role of apoptosis, mitochondrial damage, DNA damage, ROS generation, androgen receptor expression, signal transduction pathway activation, and endoplasmic reticulum (ER) stress on cytotoxicity under both NO and HO conditions.
Results: DAR had significant cytotoxicity against prostate cancer cells in vitro under both NO and HO conditions, with approximately twice as much cell killing under HO than NO conditions. DAR was a significantly more potent cytotoxin than ATO. Significant radiosensitization was observed in clonogenic assays at clinically relevant doses of radiation under both NO and HO, with the greatest magnitude of sensitization observed under HO. Mechanistic studies to date demonstrate that apoptosis is an important mechanism of DAR-induced cell death, with a greater induction of apoptosis under HO than NO conditions. Interestingly, DAR increased cellular ROS and ER stress under NO, but not HO, suggesting under HO, DAR-mediated cytotoxicity may be independent of ROS. In addition, while unrepaired DNA damage could be demonstrated in cells treated with DAR under NO conditions, DNA damage was not detectable in cell treated under HO. Addition of exogenous GSH completely inhibited DAR-induced cell death in both NO and HO, which could be secondary to either replacement of depleted glutathione (GSH) and/or effects on the membrane transporter of DAR. JNK activation occurred under both HO and NO conditions, but occurred earlier and to a greater extent under the NO conditions tested. Experiments are ongoing to better elucidate the mechanism of action of DAR under HO conditions.
Conclusions: DAR has significant cytotoxic and radiosensitizing effects against HI LAPC-4 prostate cancer cells, with the greatest effect under HO conditions. In vivo experiments will be initiated shortly to further study these effects in clinically relevant murine models of HI prostate cancer. These results could have broad potential applicability for the treatment of prostate cancer, with near term translational potential.

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Sensitization of hypoxic cells to ionising radiation by a hypoxia activated inhibitor of DNA dependent protein kinase

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Tumour hypoxia is a negative prognostic marker for patients undergoing radiation therapy. Radiation therapy acts by inducing DNA damage and DNA double strand breaks (DSB) are the primary lethal lesion caused by ionizing radiation (IR). DNA dependent protein kinase (DNA-PK) is a key holoenzyme in the non-homologous end joining (NHEJ) repair pathway which is the predominant mechanism used to repair IR induced DSBs. We have synthesized prodrugs of DNA-PK inhibitors that are bioreductively activated in hypoxic conditions and have demonstrated that these compounds can selectively sensitize hypoxic cells to IR.