A Phase Ib study of BKM120 combined with abiraterone acetate
Live imaging of cellular signaling in patient-derived tumor
Clinical implication of UGT1A1 promoter polymorphism for

Materials and Methods: We used the Eμ-Myc model of B-cell lymphoma to test pharmacological inhibitors of PI3K/AKT/MTOR signalling in combination with CX-4561 as the former pathway is known to potently regulate both transcription and translation. We investigated the effect of PI3K/AKT/MTOR inhibition on the transcription of a set of AR-regulated genes in metastatic bone biopsies. The primary study objective (NCT01741753) is to determine the safety profile and maximum tolerated dose (MTD) of BKM120 (pan-PI3K inhibitor) in combination with CX-5461 (nucleolar stress inducer). The secondary study objectives are to assess the impact of PTEN status on transcription, duration of response/time to progression in the expansion cohort, and to perform correlation studies.

Materials and Methods: The trial design involves a 14 day lead-in phase with BKM120 alone, to assess single-agent toxicity and perform correlative studies. A/P is combined with BKM120 at the end of 14 days using the standard 3+3 dose-escalation design with 3 dose levels of BKM120 (80mg, 100mg, 120mg, respectively), and participants are assessed for safety and MTD on CX-5461. The dose-escalation phase provides a rationale to combine such drugs in the clinic for the treatment of MYC driven cancer.

No conflict of interest.

A Phase Ib study of BKM120 combined with abiraterone acetate for castrate-resistant, metastatic prostate cancer

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Background: There is significant cross-talk between PI3-kinase (PI3K) pathway and androgen receptor (AR) signaling pathways, which are both critical for cell survival in castrate-resistant prostate cancer (CRPC). The primary study objective (NCT01741753) is to determine the safety profile and maximum tolerated dose (MTD) of BKM120 (pan-PI3K inhibitor) in combination with CX-5461 (nucleolar stress inducer). The secondary study objectives are to assess the impact of PTEN status on transcription, duration of response/time to progression in the expansion cohort, and to evaluate the impact of BKM120 on a PI3-kinase activation fingerprint in metastatic bone biopsies. The exploratory objective of the study is to assess the effect of BKM120 on transcription of a set of AR-regulated genes in metastatic bone biopsy samples.

Materials and Methods: The trial design involves a 14 day lead-in phase with BKM120 alone, to assess single-agent toxicity and perform correlative studies. A/P is combined with BKM120 at the end of 14 days using the standard 3+3 dose-escalation design with 3 dose levels of BKM120 (80mg, 100mg, 120mg, respectively), and participants are assessed for safety and MTD on CX-5461. The dose-escalation phase provides a rationale to combine such drugs in the clinic for the treatment of MYC driven cancer.

No conflict of interest.

Live imaging of cellular signaling in patient-derived tumor organoids

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Introduction: Colorectal cancer (CRC) patients with wild-type Kirsten-RAS (K-Ras) are more likely to benefit from monoclonal antibody therapy directed against epidermal growth factor receptor (EGFR) than patients carrying oncogenic K-Ras mutations. This finding illustrates the need to unravel the role of mutations in drug response and, most importantly, to unravel the mechanisms through which drug resistance can develop in order to define effective personalized treatment. Currently, stem cell-based CRC organoids present the most promising 3D in vitro model system to study CRC tumors: they represent patient-specific genetic make-up, allow easy genetic manipulation, long-term live imaging, and identification of different cell types. Moreover, we can directly compare responses from CRC and healthy tissue from the same patient. In the present study, we characterize a panel of patient-derived CRC organoids and we will use these to investigate mechanisms of drug-response.

Advanced imaging, biosensors and drug resistance: Lentiviral integration of fluorescently tagged Histone2B (H2B-Dendra) and state-of-the-art live cell microscopy enable us to trace individual cell migration, to monitor mitotic events and to detect apoptotic DNA fragmentation. In this way, we analyze how the organoids’ genetic make-up impacts on phenotype, growth rates and responses to stromal factors.

Furthermore, we will examine how targeted drugs affect proliferation and survival of the organoids by visualizing oncogenic signaling via the introduction of established biosensors, such as the FRET-based sensor for ERK. With these in vivo analyses, we aim to identify cellular heterogeneities within the organoids, which are expected to underlie mechanisms of drug resistance.

Perspectives: We anticipate that this study will provide patient-specific insights between mutation spectra and their influence on the cellular mechanisms of CRC behavior, as well as in their responses to drug treatment.
The pig as a model for human cancer

Dublin, Ireland, 2University of Massachusetts Medical School, Cancer tohumanoncology. Weareconfidentthatboththesepigmodelswillmakesignificantcontributionsmodeloncogenicmutantp53foundinsporadichumancancers[5]. Frequentmissensemutationinmanysporadichumancancers[4]. Wecreated syndrome. A survey of somatic p53 mutations reveals R175H as the most Somaticmutationsaffectingp53functionarepresentinmosthumancancers, responsible for FAP. At one year old the APC1311 mutation resulted in nonsensemutationsinAPCatsitesorthologoustohumangermlinemutations polyposis (FAP) [2]. We generated gene-targeted cloned pigs carrying and the inherited predisposition to colorectal cancer, familial adenomatous APC plays a vital initiating role in both sporadic colorectal cancer (CRC) [1] and the inherited predisposition to colorectal cancer, familial adenomatous polyposis (FAP) [2]. We generated gene-targeted cloned pigs carrying normal APC at sites orthologous to human germline mutations responsible for FAP. At one year old the APC1311 mutation resulted in >60 polyps in the colon and rectum. Histological and molecular analysis showed that the porcine model recapitulates all major features of early stage human FAP [3]. Somatic mutations affecting p53 function are present in most human cancers, and germline TP53 mutations are responsible for Li-Fraumeni multiple cancer syndrome. A survey of somatic p53 mutations reveals R175H as the most frequent missense mutation in many sporadic human cancers [4]. We created gene-targeted pigs carrying a latent TP53R167H mutant allele orthologous to human mutant TP53R175H that can be activated by Cre recombination to model oncogenic mutant p53 found in sporadic human cancers [5]. We are confident that both these pig models will make significant contributions to human oncology.

Reference(s)


No conflict of interest.

BCL-2 dependence in T-cell leukemia is defined by the maturation stage of the clone of origin

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Lymphoblastic Leukemia (T-ALL) is a common hematopoietic malignancy of children and adults derived from T-lymphoid cells. One subtype of T-ALL, which arises from an early T-cell precursor (ETP) cell, is associated with a poor clinical outcome with a higher risk of relapse and a poor overall survival. ETP occurs in approximately 15% of T-ALL and it is identified by surface markings and by gene expression analysis. In the field of apoptosis and cancer biology at the moment we have exciting new therapeutic agents in the variety of BH3 mimetics. Navitoclax (ABT-263) is an orally bioavailable BCL-2/BCL-XL inhibitor however it also has an on-target side effect of thrombocytopenia that has limited its use in leukemia in particular. The re-engineered BCL-2 selective inhibitor ABT-199 offers a potential treatment option for BCL-2 dependent cancers without the on-target side effects.

In this study, we utilized BH3 profiling as a functional tool to measure anti-apoptotic dependencies in T-ALL. In brief, peptides generated from the functional BH3 domain of pro-apoptotic proteins are added to cells and mitochondrial permeabilisation is measured. The peptides have selective binding for distinct anti-apoptotic proteins and therefore dependencies on anti-apoptotic proteins can be uncovered. Cell lines and primary patient samples of a more mature T-ALL subtype were found to be primarily dependent on BCL-XL. Similarly, T-ALL cell lines and primary patient derived samples are killed more efficiently by ABT-263 than by the BCL-2 selective inhibitor ABT-199, consistent with the BCL-XL dependence observed by BH3 profiling. Interestingly, ETP cases of T-ALL were found to be BCL-2 dependent by BH3 profiling and equally sensitive to both ABT-199 and ABT-263. As T-cells mature in the thymus they differentially depend on either BCL-2 or on BCL-XL. Importantly, BH3 profiling correctly predicted in vivo response of ETP-ALL patient-derived xenografts (PDX) to ABT-199 treatment. In conclusion, our study suggests that selective BCL-2 dependence can be distinguished from BCL-XL dependence in primary T-ALL cells by BH3 profiling, and that this distinction is caused by the maturation stage of the clone and has important implications for the treatment of high risk ETP-ALL.

No conflict of interest.

Plauonotil inhibits doxorubicin-induced renal cell death

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Background: Doxorubicin, an anthracyclin antibiotic, has been continuously acknowledged for its effectiveness in treatment of various human carcinomas. However, in certain cases, doxorubicin caused undesirable side effects, such as a serious degeneration of tissue component of heart and kidney. Doxorubicin-induced nephrotoxicity is a dose-limiting toxicity with presence of dramatic increase of serum creatinin and atrophy of tubular renal cells. In searching for the safe and effective compound to be used as chemoprotective agent to cease toxicity of doxorubicin on renal cells, the present study demonstrated herein that plauonotil, a Croton stellalopilosus Ohba extract, showed its potential protection against doxorubicin-induced cell death in human proximal tubule epithelial cells.

Materials and Methods: Human proximal renal HK-2 cells were pretreated with non-toxic concentrations (0–40 μM) of plauonotil for various time points (0–12 h) prior to being treated with doxorubicin. Cell viability and mode of cell death were detected through MTT and nuclease co-staining of Hoechst 33342/propidium iodide, respectively. Western blot analysis was used to evaluate the alteration of anti-apoptotic proteins, Mcl-1 and Bcl-2.

Results: Pretreatment of the cells with 40 μM of plauonotil for at least 9 h prior to doxorubicin exposure rendered cells survival against doxorubicin. Plauonotil was shown to up-regulated anti-apoptotic Mcl-1 level whereas it had no effect on Bcl-2 level. The reduction of Mcl-1 after doxorubicin treatment was shown to be closely associated with toxic action of the drug, and the increase of Mcl-1 induced by plauonotil pretreatment was able to prevent all death induced by doxorubicin. Furthermore, protective effect of plauonotil was evaluated in human lung and melanoma cells. The results indicated that plauonotil caused no significant protective effect in human lung carcinoma cells whereas it sensitized melanoma cells to the drug-induced cell death.

Conclusion: Taken together, this finding would support the development of this safety natural compound for novel application as a renoprotective drug.

No conflict of interest.

PCL-grafted chondroitin sulfate copolymers to promote dual-mediated endocytosis for enhanced anti-cancer drug delivery

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Background: Micelles are composed of a hydrophilic shell and a hydrophobic innercore. A hydrophobic anticancer drug can be loaded into the innercore to sustain the drug in blood circulation and accumulate it at tumor tissues. In this study, we developed a new method through a direct conjugation of the hydrophobic PCL and the hydrophilic CS. The copolymers self-assemble into micelles that can be used to load an anticancer drug, CPT, in the innercore. The results of dual-mediated endocytosis for enhanced anti-cancer drug delivery.

Materials and Methods: PCL was conjugated with CS via ETRA. These copolymers were analyzed using a 1H NMR spectrometer. CPT-loaded CP micelles were prepared using a dialysis method. The micelles were characterized by DLS and TEM. To determine the stability of the lactone ring of CPT by HPLC after encapsulation, micelle/CPT was suspended in PBS (pH = 7.4) with 10% FBS and incubated at 37°C. Cytotoxicities of the CPT-loaded micelles were evaluated using a MTT assay. The Annexin-V/PI dual staining assay and TUNEL assay were used to estimate the apoptosis efficiency induced by CPT or micelle/CPT. The cellular uptake of the micelles was evaluated by CLSM. The antitumor effect of micelle/CPT was evaluated in CRL-SB221 tumor bearing mice with only one tumor grafted on the right hind leg.

Results: We grafted PCL onto CS via an ATRA method. The NMR spectrum shows CS and PCL proton peaks as indicated as A and B. From this result we ensure CP has been successfully prepared. To maintain CPT in an active lactone form is an important issue for CPT circulation in bloodstream, because the antitumor efficacy of CPT in the lactone