DDIS-17. SMALL MOLECULE INHIBITORS OF C1qBP FOR THERAPEUTIC INTERVENTION IN GLIOMA

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The immune complement cascade, initiated by the protein C1q, has an important role in brain development. C1q has complement independent functions in the brain such as microglial activation and neuroprotection. However, within the aging brain, C1q protein levels can dramatically increase (almost 300-fold) resulting in neurodegenerative disease. Recently, it was reported that C1q secreted within the tumor microenvironment acts as a cancer-promoting factor through a complement independent mechanism, likely mediated by binding to its receptor C1qBP (aka gC1qR, p32, HABP1). We have demonstrated that C1qBP (p32) is upregulated in patient derived glioma stem-like cells (GSC's), and our studies were the first to demonstrate that knockdown of C1qBP in patient derived GSC's inhibits tumor growth in vitro and in vivo. Thus, inhibiting C1qBP function, has potential for therapeutic intervention. C1qBP is a multifunctional multicompartment molecule localized to mitochondria, cytosol, nucleus, and cell surface. It was shown by several groups that tumor cell proliferation could be inhibited by either an anti-C1qBP monoclonal antibody recognizing the C1q binding site, or by a tumor homing peptide (LyP-1) binding to the C1qBP site. However, the limited permeability of peptides and mAb's to cross the blood brain barrier (BBB) makes small molecule inhibitors a better approach to inhibit C1qBP in the CNS. We have employed pharmacophore modeling of C1q and LyP-1 binding to C1qBP and have compiled a list of compounds that could bind to C1qBP. We performed an initial screen of 40 compounds for direct binding to C1qBP, and tested for inhibition of C1qBP function in multiple cell-based assays. This approach has yielded a compound that binds to C1qBP and shows moderate inhibitory activity toward C1qBP. Our high throughput strategy and cell-based assays are designed to identify a small molecule inhibitor of C1qBP amenable for structure-activity relationship (SAR) for preclinical development.

DDIS-18. A NOVEL INHIBITOR OF β-CATENIN SELECTIVELY HALTS ONCOGENIC WNT SIGNALING

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β-catenin is a key player of the WNT signaling pathway; its aberrant regulation is associated with the onset and progression of numerous types of cancer, making β-catenin an attractive drug target. However, direct inhibition of β-catenin is a challenging approach toward suppression of β-catenin-driven oncogenicity. We have identified BCAT-D4, (referred to as D4 in the manuscript) a drug candidate that binds to a novel allosteric hotspot on the surface of β-catenin. It interacts with the target with nanomolar binding affinity, selectively reduces viability of human β-catenin-driven cancer cells, and activates degradation of β-catenin. We show that D4 reverses nuclear translocation of β-catenin, and inhibits stemness-promoting WNT signaling downstream of β-catenin. We report a pharmacologic approach for selective inhibition of β-catenin via a novel allosteric modulation site. We are testing applicability of D4 on brain tumors.

DDIS-19. OLAPARIB PENETRATES TUMOUR MARGINS AS WELL AS CONTRAST ENHANCING REGIONS OF GLIOBLASTOMA AT THERAPEUTIC LEVELS: INTERIM RESULTS OF THE OPARATIC TRIAL NCT01390571

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BACKGROUND: Drug delivery in glioblastoma (GBM) is challenging; poor activity may result from low biological efficacy and/or poor brain penetration. While the blood-brain barrier (BBB) is compromised in contrast-enhancing regions, little is known about BBB integrity and drug penetration in non-enhancing regions of GBM including tumour margins. Adverse clinical outcomes might reflect poor drug delivery to viable, invasive tumour cells in these regions. We evaluated tumour core and margin concentrations of olaparib (Lynparza), a small molecule inhibitor of poly(ADP-ribose) polymerase (PARP). Preclinical studies indicate that >90% PARP inhibition is achieved at 10-100 nM olaparib. METHODS: Pre-clinically, BBB penetration was assessed in MDCKII cells expressing MDR1 and by autoradiography of rats and mice treated with [14C]-olaparib. Clinically, seventeen patients with recurrent resectable GBM underwent tumour resection after four days of olaparib (tablet) dosing (100 – 400 mg daily). In three patients, additional biopsies were taken from tumour margin regions identified by 5-aminolaevulinic acid guided resection. Olaparib levels in snap frozen specimens were measured by LC-MS. RESULTS: Olaparib was a substrate for MDR1 and efflux was blocked by ketoconazole. Radioactivity was not detected in the central nervous systems of healthy rats or mice after single dose [14C]-olaparib, indicating no BBB penetration. Despite this, olaparib was detected in 49/50 tumour core specimens from 17 patients and 9/9 tumour margin specimens from 3 patients. Olaparib concentrations in tumour core specimens ranged from 138 to 1233 nM (mean 584 nM), similar to those observed in previous breast cancer studies. Tumour margin olaparib concentrations ranged from 308 to 1090 nM (mean 681 nM). Within individual patients, margin:core ratios ranged from 0.5 to 3.8 (mean 1.8). CONCLUSIONS: Olaparib is excluded from the CNS under normal conditions but reliably penetrates recurrent GBM at concentrations expected to effectively inhibit PARP. Preliminary results demonstrate similar penetration of tumour margins.

DRUG RESISTANCE

DRES-01. BONE MARROW RESPONSE AS A POTENTIAL BIOMARKER OF OUTCOMES IN GLIOBLASTOMA PATIENTS

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OBJECT: Glioblastoma (GBM) is a highly aggressive malignancy which requires a multi-disciplinary therapeutic approach of surgery, chemotherapy and radiation therapy. Treatment-related side-effects can be challenging to patient management. The most common adverse effect of chemotherapy with temozolomide (TMZ) is myelosuppression. It remains unclear whether the degree of bone marrow suppression might serve as a biomarker for outcome. The aim of the current study was to investigate whether the pattern of the degree of bone-marrow toxicity in patients treated with TMZ correlates with overall survival (OS) and MRI-based time to progression (PFS). METHODS: Complete blood counts, clinical and radiographic information were collected retrospectively from 86 malignant glioma patients who had completed both radiation therapy and at least 6 monthly cycles of chemotherapy with TMZ. RESULTS: Using a multivariate cox proportional hazard model, it was observed that treatment-induced decreases in white blood cell counts, MGMT promotor methylation, wild-type EGFR, IDH mutation, and younger patient age at diagnosis were associated with improved OS. The 2-year survival rate was 25% and 58% for patients with increases and decreases, respectively, in white blood cell counts from baseline over 6 months of TMZ treatment ($p = 0.0019$). Consistent with the literature, IDH mutation and MGMT promotor methylation were associated with improved PFS and OS. IDH mutation and MGMT promotor methylation were not correlated with changes in white blood cell counts. CONCLUSION: Decreases in white blood cell counts might serve as a potential biomarker for OS and PFS in malignant glioma patients treated with standard chemoradiation. It remains unclear whether treatment induced changes in white blood cell counts correlate with drug induced anti-tumor activity or represent an independent factor of altered systemic and tumor microenvironment. Additional studies will be needed to identify chemotherapy associated dose-dependent effects and to characterize the white blood cell subpopulations that might account for the observed effects.

DRES-02. YOUNG ADULT PATIENTS WITH ANAPLASTIC GLIOMA MAY NOT BENEFIT FROM RADIATION PLUS TMZ TREATMENT COMPARED TO RADIATION ONLY

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PURPOSE: Age is the major therapy-independent prognostic factor in malignant gliomas, but few studies have investigated treatment and survival of young adults. We aim to assess the relevance of survival and treatment in age-specific subgroups, especially younger adult patients with high-grade gliomas in a large-scale population from the Chinese Glioma Genome Atlas (CGGA). METHODS: 726 patients with confirmed anaplastic glioma or glioblastoma were enrolled in this study. Overall survival and progression-free survival were analyzed according to treat-

ment in young adults (age<50) and older adults (age≥50). RESULTS: In total, 264 of 726 of the patients were older patients (OP), and 462 were young patients (YP). Among all GBM patients and OP with anaplastic gliomas, patients assigned to RT plus TMZ had significantly longer OS and PFS than patients assigned to RT alone (P<0.05). In contrast, among YP with anaplastic gliomas, a more favorable survival benefit was not observed in the RT plus TMZ treatment group compared to the RT only group. CONCLUSIONS: We observed no survival benefit in young adult patients (age<50) with anaplastic glioma when TMZ was added to RT. Our findings should provoke discussion in relation to younger patients with anaplastic glioma who are receiving radiotherapy plus TMZ chemotherapy.

DRES-03. EXPRESSION OF DRUG RESISTANCE GENES ASSOCIATED WITH HYPOXIA IN HUMAN GLIOMA

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The brain tumor cells can survive regardless of hypoxia and undernutrition. Hypoxia is known to be causes of resistance to radio-chemotherapy, increased recurrence and poor prognosis. Recent reports demonstrated that cancer stem cells (CSCs) exist in hypoxic lesion of the tumor. In this study, we examined the mechanism of drug resistance under hypoxic condition. Most of brain tumor patients are treated by temozolomide (TMZ) as chemotherapy. The efficiency and resistance of TMZ are associated with the O6-methylguanine-DNA methyltransferase (MGMT) gene. In addition, MGMT expression is induced by hypoxia, and regulated by N-myc downstream regulated gene 1 (NDRG1). We investigated the expression of these genes in glioma cell lines (GCs), CSCs, and clinical specimens detected by molecular hypoxia imaging (PET). The expression of MGMT and NDGR1 was induced by hypoxia in GCs. Hypoxic clinical specimens also showed the increased expression of these genes. Thus, it is suggested that the improvement of hypoxia could lead to overcome the resistance of treatments.

DRES-04. TUMOR HETEROGENEITY CONTRIBUTES TO RESISTANCE TO ANTI-EGFR THERAPY IN GLIOBLASTOMA

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Glioblastoma is the most aggressive tumor type affecting the adult brain, with an overall patient survival from the time of diagnosis of about 15 months. Tumor promoting proteins such as EGFR (Epidermal Growth Factor Receptor) and its mutant form, EGFRvIII, are amplified in a subgroup of patients and have attracted attention as potential therapeutic targets. Unfortunately, kinase inhibition of EGFR or EGFRvIII has been proven to be ineffective due to resistance mechanisms that prevent the onset of cell death. We found that the presence of cytokines in the tumor microenvironment, induced by inter-clonal communication between heterogeneous tumor cell populations expressing EGFRvIII and EGFR, in addition to their previously shown promotion of glioblastoma growth, also promote resistance to EGFR-directed therapies. To identify the mechanism mediating resistance, we interrogated core pathways known to protect cancer cells from induced cell death. Results show that EGFRvIII cells expressing interleukin-6 (IL-6) promote paracrine activation of NF-kB in EGFR cells and subsequent expression of a NF-kB target gene, survivin (BIRC5), known to block cell death by attenuating caspase 3/7 activation. Demonstrating a causal link of cytokine signaling and EGFR TKIs resistance, pharmacological inhibition of NF-kB or knockdown of survivin restored sensitivity to gefitinib, erlotinib or lapatinib, both in vitro and in in vivo models of EGFR/EGFRvIII heterogeneous tumor growth. These results illustrate a novel treatment approach to enhance EGFR inhibition by uncoupling the effect of a paracrine-mediated survival pathway established by heterogeneous tumor growth.

DRES-05. TARGETING DNA REPAIR MECHANISMS IN GLIOBLASTOMA: FROM BASIC MECHANISMS TO PRE-CLINICAL ASPECTS AND PERSONALIZED THERAPY

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Despite surgical resection and genotoxic treatment with ionizing radiation and the DNA alkylating agent temozolomide (TMZ), glioblastoma remains a highly lethal cancer, due to the action of DNA repair mechanisms that drive resistance and tumour relapse. One such mechanism involves the MGMT protein which removes the alkyl group from O^6-methylguanine, the most highly cytotoxic lesion induced by TMZ. MGMT promoter methylation is observed in about 40% of GBM patients and confers a survival benefit in patients treated with TMZ. However, alkylating agent chemotherapy has hitherto been largely inefficient. While MGMT-positive patients do not benefit at all from TMZ, the survival benefit of TMZ in MGMT-negative patients is a mere 2.5 months. In addition to MGMT, several pathways are involved in the repair of TMZ-induced lesions. Understanding their molecular details and identifying potential pharmacological targets have emerged as vital tasks to improve treatment. To understand the molecular basis behind chemoresistance, tumour progression and relapse in GBM, we have measured the mRNA expression levels of a selection of DNA repair and cell cycle factors in paired, primary and recurrent GBM biopsies. Classification of the deregulated genes led to the suggestion that one route to tumour progression is associated with profound alterations in cell cycle genes as well as genes encoding crucial DNA repair factors. We are investigating our differential gene expression data further, to learn how cancer cells modulate the expression of specific pathways in response to glioblastomagenesis and genotoxic treatment, and propose novel therapeutic strategies. Lastly, we have carried out a large-scale shRNA screen of DNA repair/chromatin factors in GBM cells to identify those genes required for resistance to TMZ in vitro, as well as synthetic lethal interactions with MGMT. Promising candidates will be validated in vitro as well as in vivo, using orthotopic xenograft models of GBM.

DRES-06. COMBINATION THERAPY OF LDK378 AND INC280 (INCB028060) ENHANCES TEMOZOLOMIDE SENSITIVITY IN MGMT UNMETHYLATED GLIOBLASTOMA: PRE-CLINICAL ASSESSMENT

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Temozolomide (TMZ) therapy is the standard of care for patients with glioblastoma (GB), and resistance to this drug is at least partly modulated by the DNA repair protein O(6)-methylguanine-DNA methyltransferase (MGMT). Expression of MGMT is silenced by promoter methylation in approximately half of GB tumors, and clinical studies have shown that elevated MGMT protein levels or lack of MGMT promoter methylation is associated with TMZ resistance in some, but not all, GB tumors. Another reason for treatment failure in GB management is signal redundancy due to coactivation of several functionally linked receptor tyrosine kinases (RTKs), including anaplastic lymphoma kinase (ALK), and c-Met (hepatocyte growth factor receptor). As such, these could be attractive targets for GB therapy. Thus, we tested two novel drugs: LDK378 (highly selective anaplastic lymphoma kinase-ALK inhibitor) and INC280 (INCB028060: highly selective c-Met receptor tyrosine kinase-RTK inhibitor), aiming to overcome TMZ resistance in MGMT unmethylated GB cells in in vitro and ex vivo slice culture models. Treatments were examined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, western blot analysis, caspases assay, and PCR assay. Results obtained from our experiments demonstrated that combination of LDK378 and INC280 statistically significantly enhances the efficacy of TMZ, and also strongly inhibited the proliferation of GB cells via suppression of ALK and c-Met expression. The use of these drugs resulted in a decrease in expression of the mismatch repair (MMR) proteins, MSH6 and MLH1. Also, they induced apoptosis by modulating downstream mediators such as STAT3, AKT, and ERK. Taken together, this data indicates that co-inhibition of ALK and c-MET can enhance growth inhibitory effects in MGMT unmethylated cells and augment TMZ sensitivity in-vitro, suggesting the potential value for ALK inhibitors combined with c-MET-targeting for therapeutic benefit in MGMT unmethylated GB patients.

DRES-07. SIGNIFICANT SEX DIFFERENCES IN TEMOZOLOMIDE EFFICACY IN GLIOBLASTOMA (GBM) ARE DETECTABLE IN CELL AND PATIENT BASED EVALUATIONS

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