pathways have become the standard-of-care and have improved patient outcomes, but emergence of resistance to these agents is common. Novel therapies targeting parallel or orthogonal pathways are needed for patients with resistant tumors. Over 90% of ccRCC tumors express a mutant form of the Von Hippel-Landau protein (pVHL), an E3 ubiquitin ligase. The mutant pVHL is unable to polyubiquitinate the transcription factor hypoxia-inducible factor 2a (HIF-2a), leading to its accumulation during tumor hypoxia and promoting its activity as a tumorigenic driver. We have developed an RNAi-based approach for inhibiting HIF-2a expression using a targeted platform called Dynamic Polyconjugate (DPC), which enables efficient delivery of siRNA targeting HIF-2a directly to ccRCC tumors.

Materials and Methods: The DPC platform comprises a membrane active polymer to promote RNAi trigger endosomal release, a ligand that binds to alphaV-containing integrins on tumor cells, reversible masking to prevent polymer activity prior to endosomal compartmentalization, and an RNAi trigger to HIF-2a.

Results: Target validation was achieved by expression of an inducible shRNA to HIF-2a in established ccRCC tumors in mice, resulting in significant HIF-2a gene knockdown and tumor regression after induction. The integrin-targeting DPC (ITG-DPC) exhibits significant internalization in multiple renal tumor cell lines both in vitro and in tumor bearing mice. Treatment of nude mice harboring established orthotopic A498 ccRCC tumors with weekly injections of ITG-DPC led to >80% knockdown of HIF-2a mRNA in tumors. Furthermore, tumors from ITG-DPC treated mice exhibited statistically significant reductions in size and weight, massive apoptosis of tumor cells, reduction in the tumor-expressed VEGF-A biomarker and ablation of neovascularization as evaluated by CD31 staining.

Conclusion: These data indicate that ccRCC targeting by delivery of a potent and specific RNAi trigger to HIF-2a has the potential to significantly impact late stage ccRCC progression.

Conflict of interest: *Ownership: We are employees and stockholders of Arrowhead Research Corporation.*

354 POSTER

Phase I study of investigational oral mTORC1/2 inhibitor MLN0128: Expansion phase in patients with renal, endometrial, or bladder cancer

M. Voss[1], M.S. Gordon[2], M. Mita[3], B. Rini[4], V. Makker[5], T. Macarulla[6], D. Smith[7], E.L. Kwak[8], A. Cervantes[9], I. Puzanov[10], R. Pili[11], D. Wang[12], S. Jalal[13], S. Pant[14], M. Patel[15], R. Neuwirth[16], F. Zohren[17], J. Infante[18]. *[1]Memorial Sloan Kettering Cancer Center, Department of Medicine, Division of Solid Tumor Oncology − Genitourinary Oncology, New-York, NY, USA; [2]Pinnacle Oncology Hematology, Medical Oncology, Scottsdale, USA; [3]Cedars-Sinai Medical Center, Samuel Oschin Comprehensive Cancer Institute, Los Angeles, USA; [4]Cleveland Clinic Foundation, Department of Solid Tumor Oncology, Cleveland, USA; [5]Memorial Sloan Kettering Cancer Center, Department of Medicine, Division of Solid Tumor Oncology, New York, USA; [6]Vall d'Hebron Institute of Oncology, Oncology, Barcelona, Spain; [7]University of Michigan, Internal Medicine, Ann Arbor, USA; [8]Massachusetts General Hospital Cancer Center, Department of Medicine, Division of Hematology/Oncology, Boston, USA; [9]Hospital Clinico Universitario De Valencia, Hematology and Medical Oncology, Valencia, Spain; [10]Vanderbilt University Medical Center, Medicine, Division of Hematology-Oncology, Nashville, USA; [11]Indiana University − Simon Cancer Center, Department of Medicine, Division of Hematology/Oncology, Indianapolis, USA; [12]Henry Ford Health System, Hematology/Oncology, Detroit, USA; [13]Indiana University Melvin & Bren Simon Cancer Center, Hematology/Oncology, Indianapolis, USA; [14]Oklahoma Health Science Center − Stephenson Cancer Center, Hematology/Oncology, Oklahoma City, USA; [15]Florida Cancer Specialists/SCRI, Drug Development Unit, Sarasota, USA; [16]Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Global Statistics, Cambridge, USA; [17]Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Oncology Clinical Research, Cambridge, USA; [18]Sarah Cannon Research Institute/Tennessee Oncology, Drug Development Unit, Nashville, USA*

Background: The mammalian target of rapamycin (mTOR) pathway signaling plays a key role in cell proliferation and metabolism and is frequently dysregulated in human cancers. MLN0128 is an investigational, orally available, selective inhibitor of the mTOR multiprotein complexes mTORC1 and 2. In the dose-escalation phase of this study (NCT01058707), MLN0128 had a generally well tolerated safety profile and preliminary data suggested evidence of antitumor activity in patients with advanced solid tumors (Infante J et al, EORTC 2013, Abstract C252). Here we present data from the expansion cohorts, including patients with renal (RCC), endometrial, or bladder cancer.

Methods: Patients aged ⩾18 yrs who had failed ⩾1 prior therapy (anti-VEGF and/or TORC1 inhibitor [TORC1i] in RCC group) and had measurable disease per RECIST v1.1 were eligible. Subjects received MLN0128 by mouth 5 mg once daily (QD) or 30 mg or 40 mg once weekly (QW) in 28-d cycles. Adverse events (AEs) were graded via NCI-CTCAE v4.0; patients recorded daily pre-dose fasting blood glucose levels. Response was assessed by RECIST v1.1.

Results: At data cut-off (Mar 16), 82 patients (median age 62 yrs [30−81], 56% male) had received MLN0128 5 mg QD (n = 39), 30 mg QW (n = 17), or 40 mg QW (n = 26); 45 had RCC (20 TORC1i-naïve), 21 endometrial, and 16 bladder cancer. Patients received a median of 2 cycles (ranges were 1−12 for 5 mg QD, 1−13 for 30 mg QW, and 1−22 for 40 mg QW); 5 patients had ⩾14 cycles (all 40 mg QW). Drug-related AEs included fatigue (67%), nausea (61%), hyperglycemia (59%), stomatitis (50%), and rashes (34%). QW schedule started at 40 mg but was amended to 30 mg due to tolerability, including high rates of nausea/vomiting. In the 5 mg QD/30 mg QW/40 mg QW cohorts, 44%/35%/58% had drug-related grade⩾3 AEs; 67%/41%/77% required dose modifications or interruptions. There were no on-study deaths. Of 70 response-evaluable patients, 1 patient had a CR (40 mg QW, RCC) and 5 patients had PRs (3 at 5 mg QD: 1 endometrial, 2 RCC; 1 at 30 mg QW, RCC; 1 at 40 mg QW, RCC), for an ORR of 9%. A further 8 patients had SD for ⩾6 mos (5 at 5 mg QD: 4 RCC, 1 bladder; 3 at 40 mg QW, RCC), for an overall clinical benefit (CB) rate of 20%. The durations of CB were (QD/QW): 3.7 mos/12.4 mos for patients with RCC, 1.7 mos/3.5 mos for endometrial cancer, and 9.0 mos/1.7 mos for bladder cancer.

Conclusions: Preliminary data suggested evidence of antitumor activity in expansion cohort patients. Improved tolerability and longer duration of CB favor 30 mg QW dosing for future development.

Conflict of interest: *Ownership: Fabian Zohren: Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. Advisory Board: Martin Voss: Novartis, GSK, Bayer, Calithera. Corporate-sponsored Research: Martin Voss: Pfizer, BMS. Roberto Pili and Eunice Kwak: Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. David C. Smith, Roberto Pili, and Michael S. Gordon: Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. Other Substantive Relationships: Fabian Zohren and Rachel Neuwirth: Employee of Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited.*

355 POSTER

Targeting the STAT6 pathway to inhibit tumor-associated macrophages-induced tumor growth and metastasis in breast cancer

K. Binnemars-Postma[1], R. Bansal[1], G. Storm[1], J. Prakash[1]. *[1]University of Twente, Biomaterials Science and Technology, Enschede, Netherlands*

Background: Tumor-associated macrophages (TAMs), one of the most crucial cell types in the tumor microenvironment, contribute to tumor growth and metastasis. These pro-tumoral macrophages show an M2-like phenotype whereas their counterpart M1 macrophages have been shown to inhibit tumor growth and survival. The Signal Transducer and Activator of Transcription 6 (STAT6) pathway is the downstream signaling pathway of IL-4 and IL-13, cytokines responsible for M2 differentiation. We hypothesize that inhibition of the STAT6 pathway might be an interesting strategy to inhibit TAM (M2-like macrophage) differentiation and thereby their pro-tumorigenic activities.

Materials and Methods: Mouse RAW264.7 cells were differentiated into M2 type using IL-4 and IL-13. The STAT6 inhibitor (AS1517499, AS) was evaluated for its effects on M2 differentiation using qPCR, Western blot (pSTAT6/STAT6) and arginase activity assays. The effect of M2 cells on the migration of mouse 4T1 breast tumor cells (ATCC) was investigated using M2-conditioned medium in 4T1 scratch assay. In vivo, the 4T1-luc breast orthotopic mouse tumor model was used to evaluate the effects of AS. In tumor-bearing female balb/c mice, AS was injected (20 mg/kg i.p.) twice a week and tumor size was measured. To detect the tumor cells in the local tumor and metastasis in different organs, animals were injected with luciferine and imaged in the IVIS Lumina II system before sacrificing.

Results: Differentiation of RAW cells into M2-like phenotype was confirmed with induction of M2-specific genes (arginase-1 and mannose receptor-1) and arginase enzyme activity. Western blot analysis showed an upregulation of pSTAT6 in M2-differentiated cells compared to the control cells. Interestingly, treatment with AS (10, 100, 250 nM) dramatically inhibited the STAT6 phosphorylation in M2 cells with increasing concentrations. AS also significantly inhibited M2-induced genes and arginase activity. In contrast, AS did not show any inhibitory effects on M1 phenotype of RAW cells. Furthermore, we found that M2-conditioned medium strongly induced the migration of 4T1 tumor cells in vitro. Importantly, conditioned medium

collected from AS-treated M2 cells did not induce these paracrine effects. In vivo, treatment with AS significantly attenuated the tumor growth by about >30% and the total tumoral luciferase activity by >50%. Luciferase activities in different organs showed a reduction in metastasis in the AS group, but further histological analyses are currently being performed to confirm these data.
Conclusions: This study proposes that inhibition of the STAT6 pathway in macrophages is a vital therapeutic approach to reduce tumor growth and metastasis by inhibiting M2 differentiation (TAM formation) and M2-induced pro-tumorigenic and pro-metastatic activities.
No conflict of interest.

356 POSTER

Targeting DNA topoisomerase IIα activity with thiomaltol-based organometallic complexes

M. Novak[1], C. Hackl[1], W. Kandioller[1], M. Jakupec[1], B. Keppler[1].
[1]*University of Vienna, Institute of Inorganic Chemistry, Wien, Austria*

Background: Topoisomerase IIα is an essential nuclear enzyme involved in DNA replication, transcription, recombination and chromosome condensation. This enzyme is often overexpressed in many cancer types and became a primary target for a number of clinically important cytotoxic drugs.
Methods: The structure-activity relationships of novel thiomaltol-based organometallic complexes were studied in human cancer cell lines by the MTT assay. Compounds were studied in the DNA relaxation assay for their topoisomerase IIα inhibitory properties. Changes in cell cycle distribution induced by these substances were examined by flow cytometry using propidium iodide staining. Furthermore, we investigated by the DCF-DA assay whether thiomaltol-based organometallic complexes may alter the levels of reactive oxygen species (ROS) in cancer cells.
Results: The compounds were found to be more active in the CH1/PA-1 (ovarian teratocarcinoma) and SW480 (colon carcinoma) cells than in the more chemoresistant A549 (non-small cell lung cancer) cells. All organometallic complexes display a high capacity of topoisomerase IIα inhibition in comparison to the free ligand and inhibit the enzyme activity at a concentration of 10 μM. In addition, the ruthenium(II) methylimidazole-substituted complex is already a potent enzyme inhibitor at 2.5 μM. None of these compounds acts as a topoisomerase IIα poison since a linear DNA band (reflecting double-strand breaks as a result of stabilized TopoII-DNA cleavage complexes) is present only in the positive control (etoposide). The metal-based complexes may act as topoisomerase IIα catalytic inhibitors by blocking the ATP-binding site of the enzyme, by preventing the binding of the enzyme to DNA or by preventing the cleavage of DNA. Cell cycle studies reveal an increase of the S-phase fraction after 12 h exposure to the complexes, consistent with topoisomerase inhibition. The highest S-phase accumulation was shown for ruthenium(II) and iridium(III) methylimidazole-substituted complexes. Data of ROS experiments indicate a slight increase in ROS levels for the rhodium(III) methylimidazole-substituted complex by a factor of 2.7 but no significant effect for other tested substances.
Conclusions: The results suggest that topoisomerase IIα is a target for thiomaltol-based organometallic complexes. In addition, these metal-based complexes show a significantly higher enzyme inhibitory capacity compared to the free ligand. The reported findings make organometallic compounds based on thiomaltol derivatives promising candidates for the development of anticancer agents with topoisomerase IIα inhibitory properties.
Acknowledgment: Authors are indebted to the Johanna Mahlke née Obermann Foundation for financial support.
No conflict of interest.

357 POSTER

Toxicity remains the key determinant of recommended phase II dose (RP2D) of anticancer agents despite increasing use of non-toxicity endpoints

A. Hansen[1], N. Cook[1], A. Eitan[1], L. Siu[1], A. Razak[1]. [1]*Princess Margaret Hospital, DMOH − Drug Develpment Program, Toronto, Canada*

Background: The recommended phase II dose (RP2D) of anticancer agents is determined traditionally by dose limiting toxicities. Non-toxicity parameters such as pharmacokinetics (PK), pharmacodynamics (PD) and efficacy can also be utilized to identify RP2D. This methodology is thought to be relevant especially in evaluation of molecularly targeted agents (MTA).
Methods: A systematic review identified all monotherapy phase I studies of MTA in cancer, published between 2001 and 2013. Dose, schedule and determinants of RP2D were collected from each study. A supplementary search of the FDA website identified the licensed dose for drugs with regulatory approval. Descriptive statistics summarized the data, and logistic regression analysis was used to determine predictors for the RP2D matching the final approved dose.
Results: The search identified 4175 records, of which 255 studies evaluating 181 MTA were included. RP2D determined by multiple endpoints were reported in 100 (39%) trials with toxicity/PK being the most common combination (44%) followed by toxicity/PK/PD (10%). There has been increasing use of PD and efficacy endpoints to define RP2D over time (trend p 0.04 and p 0.006 respectively). Toxicity was the sole factor used in 54 (21%) studies, whereas PK (4%), PD (<1%) and efficacy (0%) were used rarely as a single parameter to define RP2D. A total of 28 drugs (15%) evaluated in 66 phase I trials subsequently received regulatory approval for use in solid tumors. In 18 of these agents (64%) evaluated in 28 trials (42%), the RP2D was the same as the subsequent FDA approved dose. The majority (71%) of these trials used multiple endpoints to establish RP2D with only 9% of trials not using toxicity. For agents with a discordant RP2D and approved dose (7/28), 60% of these trials used multiple endpoints. Use of PK, PD or multiple endpoints to define RP2D showed higher odds of predicting the final approved dose, but these associations were not significant.
Conclusions: The use of non-toxicity endpoints to determine RP2D has increased over time, but their utility is in combination with, rather than in lieu of toxicity. Further research is required to quantify the incremental value of PK, PD and efficacy endpoints in addition to toxicity as determinants of the RP2D. These efforts will aid in designing trials with endpoints that define an optimal biological dose.
No conflict of interest.

358 POSTER

Anticancer agent derived life-threatening skin toxicities based on spontaneous reporting data in Japan

R. Tanaka[1,2], K. Yonemori[3], A. Hirakawa[4], F. Kinoshita[4], N. Yamazaki[2], M. Fujimoto[1], K. Tamura[3], Y. Fujiwara[3]. [1]*University of Tsukuba, Department of Dermatology, Tsukuba, Japan;* [2]*National Cancer Center Hospital, Dermatologic Oncology Division, Tokyo, Japan;* [3]*National Cancer Center Hospital, Breast and Medical Oncology Division, Tokyo, Japan;* [4]*Nagoya University Hospital, Biostatistics and Bioinformatics Section, Center for Advanced Medicine and Clinical Research, Nagoya, Japan*

Background: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous and mucosal adverse drug reactions which are rare and potentially life-threatening.
Although drugs are almost always implicated in both, the connection to anticancer agents is not well understood. Herein is an analysis of the clinical characteristics and profile of SJS or TEN induced by anticancer agents in the Japanese population.
Material and Methods: Searching spontaneous reporting data from the Pharmaceuticals and Medical Devices Agency (PMDA) Medical Safety Information yielded 9253 cases of SJS or TEN out of 289494 reports. Identified from these were: age, sex, skin toxicity type (SJS/TEN), toxicity onset date, outcome, follow-up termination date or date of death, and the suspected causal drugs' name, intended purpose and initiation date. In addition, the suspected drugs were categorized by efficacy. Finally, with 8921 cases, factors such as age, sex, and time to onset of SJS/TEN were analyzed using a chi-square test and logistic regression and cox regression models.
Results: Of 8921 SJS/TEN cases, 485 cases (5.4%) identified anticancer agents as the cause: of these, 53 cases (11%) were fatal. Multivariate analyses showed cases using anticancer agents had significantly lower incident risk of death compared to non-anticancer agents (HR = 0.6, P = 0.0006). There were 384 cases (79%) of SJS and 101 cases (21%) of TEN. Males slightly outnumbered females (55% vs 45%).
The median initiation duration to onset of SJS/TEN was 18 days, which was significantly longer than that of the non-anticancer agent group (11 days, P < 0.0001). Multivariate analyses also showed statistical significance (HR = 0.6, P < 0.0001). Moreover, 15% of cases within the anticancer agent group developed SJS/TEN more than 70 days after initiation of the suspected causal drug; significantly higher than the non-anticancer agent group (7%, P < 0.0001).
Conclusions: More than 5% of cases with life-threatening skin toxicities, such as SJS or TEN, were caused by anticancer agents. The longer duration to the onset of skin toxicity, compared to other types of drugs, raises the need for vigilance and ongoing monitoring for this side-effect when using anticancer agents.
No conflict of interest.