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MEETING REPORT

23RD AACR-NCI-EORTC INTERNATIONAL CONFERENCE ON MOLECULAR TARGETS AND CANCER THERAPEUTICS

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SUMMARY

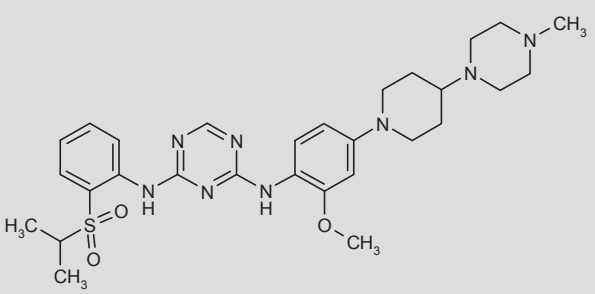
The 23rd AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics was held in San Francisco, California, on November 12-16, 2011. Over 3,000 researchers were expected to attend, with 750 abstracts being presented in oral and poster sessions. Themes addressed during the meeting included rational drug development for targeted cancer drugs, mechanism-based targeted combination therapies, pharmacogenetics, immunotherapy, epigenetics and cancer stem cells. This report focuses on some of the highlights from the poster sessions during the meeting.

Key words: Monoclonal antibodies – Protein kinase inhibitors – Combination therapies – Modified formulations

PART I

ASP-3026: EML4-ALK-dependent tumor models

The oncogenic fusion kinase EML4-ALK was first identified in a non-small cell lung carcinoma (NSCLC) patient in 2007. Approximately 5% of NSCLC patients are thought to possess this translocation, and in most of these, *EML4-ALK*, *EGFR* and *KRAS* gene mutations are known to be mutually exclusive. Proof that EML4-ALK is a therapeutic target for a subset of NSCLC patients has been provided by the impressive effect of the ALK/c-Met dual inhibitor crizotinib (Xalkori®) in ALK-positive NSCLC patients in clinical trials. However, the EML4-ALK Leu11196Met mutation, known as the gatekeeper mutation because of its location at the bottom of the binding pocket, has been reported to confer resistance. ALK inhibitors that effectively inhibit the resistant mutant may be of clinical benefit. Astellas Pharma's Sadao Kuromitsu presented data from studies investigating the efficacy and pharmacokinetics of the selective ALK tyrosine kinase inhibitor **ASP-3026** in *EML4-ALK* transgenic mice, as well as studies determining its efficacy against the EML4-ALK Leu11196Met mutation. The growth of human NSCLC NCI-H2228 cells endogenously expressing EML4-ALK in NOD SCID mice was inhibited by 1 mg/kg ASP-3026 once daily; the compound showed a dose-dependent increase in tumor regression at 3-30 mg/kg once daily. In hEML4-ALK transgenic C57BL/6 mice, ASP-3026 (10-100 mg/kg) caused a significant and dose-dependent regression of tumor nodules at day 7 of dosing. At day 11, a dose of 10 mg/kg did not cause a significant regression of tumor nodules; the effect was not determined for a dose of 100 mg/kg. However, the dose of 30 mg/kg p.o. once daily had a significant effect; following treatment, the concentration was 200-fold greater in tumor tissue (8005 ng/g) compared with



ASP-3026

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plasma (43 ng/mL). ASP-3026 and crizotinib exhibited IC_{50} values of 32 and 51 nmol/L, respectively, against ALK kinase with the EML4-ALK Leu11196Met gatekeeper mutation; the respective values were 10 and 5 nmol/L against wild-type ALK kinase. The viability of EML4-ALK Leu11196Met and wild-type EML4-ALK cells was decreased by ASP-3026 with IC_{50} values of 163 and 42 nmol/L; crizotinib showed corresponding values of 413 and 45 nmol/L. In nude mice bearing EML4-ALK Leu11196Met and wild-type EML4-ALK tumors, ASP-3026 (100 mg/kg) induced tumor regression of 69% and 81%, respectively, compared to 10% and 67%, respectively, for crizotinib.

Phase Ib results for U3-1287 in combination with erlotinib

The fully human anti-HER3 monoclonal antibody (MAb) **U3-1287** (AMG-888; Daiichi Sankyo/Amgen) was previously shown to demonstrate synergistic activity in combination with anti-epidermal growth factor receptor (EGFR) inhibitors in preclinical models of cancer. Jennifer Tseng from the MD Anderson Cancer Center Orlando presented data from the phase Ib portion of a phase Ib/II trial of U3-1287 in combination with the EGFR inhibitor erlotinib (Tarceva®). In a first-in-human study of U3-1287, no dose-limiting toxicities (DLTs) were observed at 20 mg/kg every 2 weeks, which was the highest dose tested. Furthermore, in non-human primates no treatment-related toxicities were observed up to the highest dose of U3-1287 tested (200 mg/kg/week). Based on these findings, patients received 150 mg/day erlotinib p.o. and 18 mg/kg U3-1287 i.v. every 3 weeks in the dose de-escalation phase Ib portion of the trial. It was planned that sequential cohorts would receive de-escalating doses of U3-1287 in the event that 18 mg/kg was not well tolerated. The primary objectives of the study were to determine the recommended phase II dose of U3-1287 for combination with erlotinib and to assess the safety and tolerability of the combination in advanced or metastatic NSCLC patients. No DLTs were observed. In two patients, six grade 3 adverse events (AEs) were observed, which included diarrhea, fatigue, pain, blood creatinine increase, dehydration and headache. With the exception of diarrhea and dehydration, which were considered to be related to erlotinib treatment, all grade 3 AEs were considered to be unrelated to treatment and no grade 4 or 5 AEs were observed. Following drug administration, all patients (N = 7) tested negative for human anti-human antibody formation. Progressive disease was observed in three patients at the week 6 visit. At this time, 4 patients had stable disease, and the duration of stable disease was 69 (at data cut-off date), 74 (at data cut-off date), 87 and 90 days. It was concluded that the combination of U3-1287 and erlotinib was generally well tolerated. Based on these results, the recommended phase II doses of U3-1287 and erlotinib were 18 mg/kg every 3 weeks and 150 mg/day, respectively. The phase II portion will involve high- and low-dose arms for U3-1287 and a placebo arm. Pharmacokinetic data from the phase I portion would be reported subsequently with phase II data.

ARQ-092: an ATP-independent protein kinase Akt inhibitor

Small-molecule inhibitors of protein kinase Akt are beginning to be considered for the potential treatment of cancers that exhibit dysregulated signaling in the protein kinase Akt pathway. Thomas C.K. Chan (ArQule) explained that **ARQ-092** (ArQule/Daiichi Sankyo) had a K_d value of 0.28 nM for binding to inactive, unphosphorylated c-Akt, and respective IC_{50} values of 2.9, 9.3 and 17 nM for c-Akt, Akt-2 and Akt-3. In a screen against 387 kinases, only 3 kinases, including MAP/microtubule affinity-regulating kinase 4 (*MARK4*; IC_{50} = 129 nM), MAP/microtubule affinity-regulating kinase 3 (*MARK3*; IC_{50} = 173 nM) and serine/threonine-protein kinase MARK1 (IC_{50} = 180 nM), were inhibited by ARQ-092 within 100-fold of its protein kinase Akt IC_{50} value. The IC_{50} value of ARQ-092 for protein kinase Akt inhibition did not increase markedly in the presence of increasing ATP concentrations; in contrast, there was an approximately 15-fold change in protein kinase Akt IC_{50} values for the c-Akt inhibitor A-674563 over an ATP concentration range of 0-1000 μM. In human metastatic endometrial adenocarcinoma AN3 CA cells, ARQ-092 exhibited EC_{50} values of 40 and 62 nM, respectively, for inhibition of the phosphorylation of Ser473 and Thr308; phosphorylation of the downstream proline-rich AKT1 substrate 1 (PRAS40; *PRAS40*) was inhibited with an EC_{50} value of 312 nM. The growth of a variety of cancer cell lines with aberrant protein kinase Akt signaling, including those originating from breast, prostate, ovarian and endometrial tissue, was inhibited with GI_{50} values ranging between 0.13 and 4.2 μM. In the AN3 CA and human breast ductal carcinoma BT-474 tumor xenograft models phosphorylation of protein kinase Akt and PRAS40 was inhibited following a single oral dose of ARQ-092. Furthermore, the compound administered p.o. once daily for 10 days caused a marked suppression in the growth of AN3 CA tumor xenografts. Based on the compound's pharmaceutical properties, clinical development was being planned.

In vivo safety profile of TAS-115

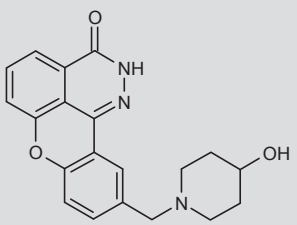
It was recently shown that malignant cancer phenotypes, including angiogenesis and metastasis, are modulated in a complementary way by proto-oncogene c-Met and vascular endothelial growth factor receptor (VEGFR). As VEGFR inhibitors have recently been associated with toxicity that requires dose modification, including interruptions and discontinuations, this c-Met/VEGFR complementary activity is an attractive alternative approach to the treatment of some cancers. Masanori Kato from Taiho Pharmaceutical presented preclinical data for the dual c-Met and VEGFR inhibitor **TAS-115**. In a biochemical assay, the compound exhibited IC_{50} values of 32 and 30 nM, respectively, for c-Met and VEGFR-2. In contrast, foretinib (GSK-1363089; GlaxoSmithKline), sunitinib (Sutent®) and crizotinib demonstrated IC_{50} values of 68 and 130 nM, > 3,000 and 18 nM, and 16 and > 3,000 nM, respectively, for these targets. In human gastric carcinoma MKN45 cells, TAS-115 demonstrated an IC_{50} value of 8 nM for c-Met-dependent growth, while in VEGF-stimulated human umbilical vein endothelial cells (HUVEC) it had an IC_{50} value of 3 nM for VEGF-dependent growth. TAS-115 selectively inhibited c-Met- and VEGF-dependent growth; cellular IC_{50} values in the absence of c-Met and VEGF supplementation were > 20 μM. There was a 2,000-fold difference in IC_{50} values in the presence and absence of both c-Met and VEGF supplementation. This difference was between 10- and 100-fold for foretinib, sunitinib and crizotinib. In the human stomach carcinoma Hs 746T xenograft model, treatment with the compound (200 mg/kg/day x 14 days) resulted in complete regression of c-Met-amplified tumor by inhibition of the c-Met signal cascade. In contrast, sunitinib (40 mg/kg/day x 14 days) treatment

resulted in partial suppression of tumor growth. Under the same dosing conditions, in a human gastric cancer AZ-521 xenograft model TAS-115 suppressed non-c-Met-expressed tumor growth via VEGFR-2 inhibition; this suppression was greater than that induced by sunitinib. In vivo, highly potent antitumor activity was demonstrated by the compound against c-Met-positive and -negative xenografts. In more than 20 xenograft models, the compound had an ED_{50} value between 3 and 25 mg/kg. In mice and rats, TAS-115 had a therapeutic index ([AUC of MTD]:[AUC of ED_{50}]) of > 27.5 and > 10; in contrast, the respective values for foretinib, sunitinib and crizotinib were 2.0 and 0.9, 1.6 and 2.5, and 1.5 and 1.6. In a longer-term efficacy study in a human gastric cancer SC-4 xenograft model, TAS-115 (200 mg/kg/day x 42 days) exhibited maintained inhibition of tumor growth and a prolonged time to progression compared with sunitinib (40 mg/kg/day, 2 x 14 days) and foretinib (6.3 mg/kg/day x 42 days); in this model, at 12.5 mg/kg/day foretinib one animal died on day 19. At the time of presentation, a phase I trial of TAS-115 was ongoing.

PART II

First clinical data reported for E-7016 and MEK-162

The AACR conference saw the presentation of the first clinical data for several new drugs, including phase I data for Eisai's oral poly [ADP-ribose] polymerase 1 (PARP-1) and 2 (PARP-2) inhibitor **E-7016** (GPI-21016). Alain Mita (Cancer Therapy and Research Center) presented results from an open-label, multicenter, dose-escalation study in patients with melanoma or advanced solid tumors. Subjects received E-7016 (4, 8, 16 or 32 mg/kg/day orally on days 1-7) alone or in combination with temozolomide (Temodar®) (150 mg/m^2/day orally on days 1-5) on a 28-day dosing cycle. The primary objective of the trial was to establish any DLTs and determine the maximum tolerated dose (MTD). Patients (N = 12) with carcinomas of the breast, ovary, kidney, lung, colon, uterus and nasopharynx were enrolled in the trial. At a dose of 8 mg/kg, three patients receiving E-7016 showed DLTs of tachycardia, hypotension and presyncope. As a result, the dose was reduced and the MTD was established at 4 mg/kg. The most common AEs were fatigue, nausea and vomiting. One grade 4 AE of hyponatremia was observed, but no serious AEs occurred. One patient achieved stable disease for > 6 months, two patients showed stable disease for < 6 months, four patients showed progressive disease and five patients were non-evaluable. The disease control rate was determined to be 25%. Pharmacodynamic data found PARP inhibition and increased DNA damage occurred in peripheral blood mononuclear cell (PBMC) patient samples up to 10 hours after treatment with both the drug alone and in combination with temozolomide. A phase II trial of E-7016 in combination with temozolomide is planned in patients with advanced melanoma.

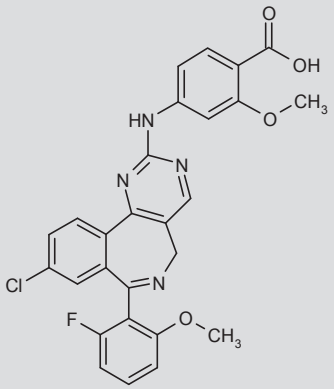


E-7016

Clinical data from a phase I trial of the orally available dual specificity mitogen-activated protein kinase kinase 1 (MEK-1) and 2 (MEK-2) inhibitor **MEK-162** (ARRY-162; Novartis) were discussed by Johanna Bendell (Sarah Cannon Research Institute). An open-label, dose-escalation trial was conducted to establish the MTD and safety profile of the compound in patients (N = 19) with advanced solid tumors. Pharmacokinetic data showed that the exposure of MEK-162 was dose-proportional, with an average maximum concentration reached within 2 hours of dosing. At all dose levels, mean plasma concentrations were maintained above the in vitro IC_{50} for cell proliferation. Of three evaluable patients, two in the 80-mg b.i.d. cohort experienced DLTs of central serous-like retinopathy and dermatitis acneiform, and the MTD was determined to be 60 mg b.i.d. The most common AEs were rash, diarrhea, nausea, vomiting, stomatitis and peripheral edema. Grade 3/4 treatment-related AEs included rash, palmar–plantar syndrome, chorioretinopathy and increased creatine kinase. Of 17 evaluable patients who demonstrated clinical antitumor activity, 15 had a response evaluation criteria in solid tumors (RECIST) target lesion at baseline. The overall best response was achieved by 1 patient who had a partial response for a duration of 10.2 months. Stable disease was achieved by nine patients with a median duration of 3.9 months. A trial expansion is currently under way in patients with biliary cancer or *KRAS*- or *BRAF*-mutant metastatic colorectal cancer to further explore toxicity and the pharmacokinetic profile of MEK-162.

Millennium and Myrexis report on modified formulations of existing pipeline compounds

As a result of the economic downturn and the low level of novel compounds that make it to the market, pharmaceutical companies are increasingly seeking to add value to their portfolio through the development of different formulations of existing drugs. Gerald Falchook (MD Anderson Cancer Center) presented results from a phase I study of an oral solution formulation of Millennium Pharmaceuticals' and Takeda Pharmaceuticals' Aurora kinase A inhibitor **alisertib** (MLN-8237), which was compared with the oral powder in capsule formulation. It is hoped that the oral solution will be better suited for patients unable to swallow solid formulations, such as the pediatric population and patients with feeding tubes. The open-label, multicenter trial (NCT00962091) enrolled 19 patients with advanced solid tumors, with 14 patients participating in the 2-way crossover part of the trial. After a single dose, the median t_{max} for absorption was 1 and 2 hours, respectively, for 25 mg oral solution and 50 mg powder in capsule formulation. The mean half-life for the same doses was 14 and 16 hours, respectively. The relative oral bioavailability of the oral solution formulation was approximately 135% compared with the powder in capsule formulation. In the powder in capsule to oral solution cohort, DLTs of grade 4 febrile neutropenia and thrombocytopenia were seen in two patients, both events being reversible. The most common AEs were fatigue, nausea



Alisertib

and diarrhea. A total of five patients achieved the best response of stable disease and the median duration of response was 4.5 months. The taste of oral solution alisertib was rated tolerable.

Preclinical pharmacokinetics and antitumor efficacy of Myrexis' **MPC-8640**, an oral prodrug of MPC-9528, a nicotinamide phosphoribosyltransferase inhibitor, were presented by the company's Robert Carlson. Different dosing schedules were evaluated in athymic mice with human fibrosarcoma HT-1080 tumor xenografts. Tumor regression was induced by b.i.d. dosing for 2 weeks and the maximal response was achieved after 1 week, with tumor growth inhibition seen at 6 mg/kg and significant regression at 10 mg/kg. No difference was observed between 7 or 14 days of dosing. When mice were dosed at 30 mg/kg once daily for 3-4 days, 24 mg/kg once daily for 4-7 days or 36 mg/kg once daily for 5-7 days, it was determined that at least 5 consecutive days of once-daily dosing were required for maximal tumor response. Antitumor activity observed after 7 days of once-daily dosing was maintained for at least 1 week thereafter. At 90 mg/kg once daily, 98% of mice survived for 1 week compared with a survival rate of 60% at > 90 mg/kg. Objective response and cure rates were high. It is believed that MPC-8640 monotherapy will demonstrate antitumor activity in clinical trials.

Astex's pyruvate kinase activator program shows promise in preclinical lung cancer studies

Astex Pharmaceuticals is investigating a program of pyruvate kinase modulators (PKMs), which includes compounds that activate the M2 splice form of pyruvate kinase (PKM2; *PKM2*) and other compounds that inhibit the M1 splice form of pyruvate kinase (PKM1; *PKM1*). The company's Mark Parnell presented preclinical data from the small-molecule PKM2 activator program. Tumor cells use PKM2 inhibition to accumulate biomass through the use of glycolysis intermediates. In a biochemical assay, two compounds, **A** and **B**, were found to have EC_{50} values of 62 and 31 nM, respectively, compared with 23 nM for fructose-1,6-bisphosphatase. The drugs also increased PKM2 affinity for phosphoenolpyruvate, but not ADP. In human lung carcinoma A549 cells, the compounds showed respective EC_{50} values of 460 and 150 nM, and 300 and 63 nM in human NSCLC NCI-H1299 cells. Cell culture studies showed that the compounds induced tetramer formation of PKM2 and inhibited the growth of A549 and NCI-H1299 cells (IC50 = 1 and 9.6 μM, respectively). In an A549 mouse xenograft model, administration of compound A (50 mg/kg every 5 days for 5 weeks) resulted in a tumor size that was 54% that of control at day 35. An increase in pyruvate was found in tumor cells and no significant weight loss or toxicity was observed.

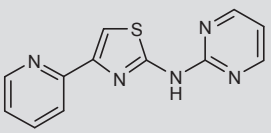
PART III

Genentech's R3Mab shows synergy with gemcitabine and carboplatin in bladder cancer models

Fibroblast growth factor receptor 3 (FGFR-3) controls cell proliferation, differentiation and survival, and mutational activation of the receptor has been reported in 50-60% of low-grade and 15-20% of muscle-invasive bladder cancer. Hao Li (Genentech) presented preclinical data on the company's **R3Mab** (presumed to be RG-7444, MFGR-1877S), a humanized MAb targeted against FGFR-3. To confirm overexpression of the receptor in bladder cancer cells, immunohistochemistry studies were conducted, which found that 8 of 13 samples overexpressed FGFR-3. In a human bladder carcinoma RT-112 xenograft model, R3Mab reduced the number of Ki67-positive cells in the tumor and inhibited FGFR-3 signaling. Single-agent efficacy was seen when the MAb was dosed twice a week in bladder carcinoma BFTC-905, UMUC-15 and UMUC-1 xenografted mice, with tumor growth inhibition (TGI) of 58%, 63% and 74%, respectively. Synergistic activity was also observed when R3Mab was administered in combination with gemcitabine and carboplatin in both UMUC-1 and bladder carcinoma RT4 tumor xenograft models. At that time, a combination treatment strategy was being planned.

Enantigen uses antiviral data to develop HBF-0079 for HCC

Enantigen is building on its research on preventing and treating infectious viral disease with the investigation of **HBF-0079** for hepatocellular carcinoma (HCC), of which 60% of cases occur as a result of hepatitis B virus (HBV) infection. Data on HBF-0079, a disubstituted aminothiazole discovered through high-throughput screening (HTS) of 85,000 small-molecule compounds, were presented by Andrea Cuconati (Institute for Hepatitis & Virus Research). In the human hepatocellular HuH-7, Hep 3B, HepaRG, MHCC97H and MHCC97L cell lines, the compound showed a CC_{50} value of 0.98,



HBF-0079

2.66, 7.65, 33.67 and 38.91 mM, respectively, but weak activity in other non-HCC cell lines. Intraperitoneal or intratumoral administration of HBF-0079 at 8.6 mg/kg in a murine Hep 3B xenograft model showed suppressed tumor growth compared with control. In vitro studies demonstrated that the compound induced the accumulation of HuH-7 cells in a sub-G_1 population, as well as modulating cell growth and antiapoptotic signaling through protein kinase Akt and serine/threonine-protein kinase mTOR. It is believed that the compound works through the inhibition of a receptor tyrosine kinase.

CureFAKtor's small-molecule compounds target FAK–VEGFR-3 interaction site

Priyanka Agharkar (Roswell Park Cancer Institute) presented data from CureFAKtor Pharmaceuticals' program of small-molecule focal adhesion kinase (FAK) inhibitors that target the FAK–VEGFR-3 interaction site. One compound, **C4**, was identified and then modified, and the resulting analogues (**C9**, **C10**, **C9A** and **C9B**) were evaluated for antitumor activity in six pancreatic cell lines, in which the analogues significantly reduced cell survival. One compound, C9, showed enhanced specificity and disrupted the binding of FAK–VEGFR-3, as well as inhibition of downstream signaling and apoptosis induction. In the human pancreatic carcinoma MIA PaCa-2-Luc murine tumor xenograft model, doses of 20 and 50 mg/kg of C9A displayed TGIs of 25% and 54%, respectively, while C10 (20 mg/kg) had a TGI of 63%. In a second MIA PaCa-2-Luc xenograft study, oral and intraperitoneal administration of C4 resulted in TGIs of 15% and 37%, respectively, while C6 and C9 showed respective TGIs of 50% and 44%, and 47% and 58%.

IMP-04297 demonstrates strong inhibition of PARP-1 activity

The therapeutic efficacy of DNA-damaging anticancer agents is increased by PARP-1 inhibitors; furthermore, these inhibitors are cytotoxic to BRCA1- and BRCA2-deficient cancer cells. In a recent phase II study, it was shown that breast cancer is effectively treated with the PARP-1 inhibitor olaparib (AZD-2281; AstraZeneca). IMPACT Therapeutics' Sui Xiong Cai presented in vitro and in vivo data for **IMP-04297**, which resulted from efforts to discover and develop novel PARP-1 inhibitors. IMP-04297 and olaparib demonstrated respective IC_{50} values of 1.2 and 4.9 nM against PARP-1. In a cell viability assay in human colon adenocarcinoma SW620 cells, IMP-04297 (GI_{50} = 2.7 nM) was 60-fold more potent than olaparib (GI_{50} = 158.9 nM). In a caspase-based apoptosis assay in human breast ductal carcinoma T-47D cells, IMP-04297 had an EC_{50} value of 2.3 nM when combined with the DNA-damaging agent methyl methanesulfonate, compared with an EC_{50} value of 17.4 nM for olaparib; however, IMP-04297 exhibited no activity in this assay when administered alone. In a cell viability assay employing BRCA2-deficient human pancreas adenocarcinoma Capan-1 cells, IMP-04297 and olaparib had respective IC_{50} values of 460.7 and 16.53 nM. In mice, IMP-04297 (10 mg p.o.) had an oral bioavailability of 47% and $AUC_{(0\text{-}t)}$, half-life, t_{max}, volume of distribution, clearance and C_{max} values of 34,9372 μg/L.min, 155 minutes, 15 minutes, 6.40 L/kg, 0.029 L/min/kg and 2357 μg/L, respectively. The corresponding values when IMP-04297 (10 mg) was administered intravenously were 74,2912 μg/L.min, 53.0 minutes, 5 minutes, 1.03 L/kg, 0.013 L/min/kg and 20,400 μg/L. In a B16F10-C57 melanoma model, the anticancer effect of the DNA-damaging agent temozolomide was potentiated by IMP-04297. A small body weight reduction was caused by both IMP-04297 (1.0 mg/kg) and olaparib (20 mg/kg); at these doses, IMP-04297 was considered to be more effective than olaparib. In a human breast carcinoma MX-1 model, good efficacy was demonstrated by IMP-04297 (1.0 mg/kg) and this was markedly greater than that demonstrated by olaparib (20 mg/kg) in combination with temozolomide. At this dose, IMP-04297 in combination with temozolomide caused a small reduction in body weight between days 6 and 12 after treatment. At the time of presentation, a patent application had been filed and it was planned that preclinical studies would be finished for an IND filing to be made in 16-18 months.

GBR-401 – a humanized anti-CD19 antibody

Jonathan Back (Glenmark Pharmaceuticals) presented on **GBR-401**, a human MAb directed against domain 2 of the human B-lymphocyte antigen CD19, as a B-cell-depleting agent for the potential treatment of B-cell malignancies. GBR-401 was shown to bind to CD19 on Ramos cells, which are CD19-positive and HER2-negative. In contrast, the compound did not bind to human breast adenocarcinoma SK-BR-3 cells, which are HER2-positive and CD19-negative. In CD19-positive tumor cells, following treatment with GBR-401 (0.001-1 μg/mL) there was a rapid onset of apoptosis, which was concentration-dependent. In the CD19-positive Ramos tumor cell line, GBR-401 inhibited proliferation in a concentration-dependent manner and this effect was greater than that observed for rituximab (Rituxan®, MabThera®). Antibody-dependent cell-mediated cytotoxicity was increased in a concentration-dependent manner by GBR-401 in Raji cells (CD19-positive), and this effect was greater and more potent than that observed for the comparator rituximab. Also in Raji cells, GBR-401 exhibited little or no complement-dependent cytotoxicity, whereas rituximab, which was used as a positive control, had a specific cytotoxicity value of > 60%. In PBMCs from healthy subjects, GBR-401 depleted human B cells in a concentration-dependent manner more potently and to a greater extent than rituximab. In a human PBMC graft model in SCID mice, GBR-401 (0.1 and 0.5 mg/kg) significantly depleted human B cells compared with an isotype control, and exhibited an EC_{50} value of approximately 0.02 mg/kg; the B-cell depletion induced by rituximab in this model was not considered to be significant. These combined findings were thought to provide a premise for the clinical testing of GBR-401 in patients with CD19-positive hematological malignancies.

FLT3 kinase inhibitors for the treatment of AML

Receptor-type tyrosine-protein kinase FLT3 is a promising therapeutic target for acute myeloid leukemia (AML), which represents approximately 90% of all adult acute leukemias. It is overexpressed in more than 50% of AMLs, and in approximately 35% of patients, mutations in the kinase result in constitutive activation. Hee Kyu Lee (Oscotec) presented data for **G-749** (Genosco/Oscotec), which was shown to have respective IC_{50} values of 0.2 and 0.3 nM for wild-type FLT3 and FLT3 Asp835Tyr. At these same two targets, quizartinib dihydrochloride (AC-220; Ambit Biosciences/Astellas Pharma) and midostaurin (PKC-412; Novartis) exhibited IC_{50} values of 8.8 and 28.2 nM, and 15.4 and 24.2 nM, respectively. From a screen of 340 kinases, G-749 was shown to be highly selective for FLT3. The ki-

nases Aurora kinase B, VEGFR-3 and Mer, which all play a role in AML, were also inhibited by the compound. G-749, quizartinib dihydrochloride and midostaurin all inhibited FLT3 autophosphorylation in a concentration-dependent manner, with IC_{50} values of 2.2, 12.0 and 7.2 nM, respectively. In a cell viability assay, G-749 was selective for the human leukemia cell line MV-4-11 (FLT3-ITD), exhibiting an IC_{50} value of 2.1 nM, compared with > 1000 nM for both human erythroleukemia HEL and human chonic myelogenous leukemia K-562 cell lines. In contrast, midostaurin was multipotent and toxic, demonstrating respective IC_{50} values of 1.8, 6 and 253 nM for these three cell lines. In murine pro-B-cell BaF3 cells transfected with a range of FLT3 mutants, G-749 exhibited superior cell viability-inhibitory activity compared with quizartinib and midostaurin. Furthermore, there was a high correlation between FLT3 inhibition and the cell viability results. G-749 was shown to induce apoptosis in a concentration-dependent manner and to inhibit the activated downstream effectors p-STAT5, p-ERK1/2 and p-AKT. Oral treatment with the hydrochloride salt of G-749, G-801 (30 mg/kg), resulted in tumor regression in a murine MV-4-11 tumor xenograft model. Furthermore, once dosing was discontinued there was no tumor relapse for 4 weeks. In a human plasma milieu, the compound demonstrated strongly sustained inhibition of p-FLT3 (IC_{50} = 9.9 nM) compared with quizartinib (IC_{50} = 52.8 nM) and midostaurin (IC_{50} > 1000 nM); furthermore, in a high FLT3 ligand milieu, which is one of the proposed drug resistances, the compound exhibited strongly sustained p-FLT3 inhibition in comparison with the other drugs. In primary AML cells from FLT3-ITD-positive and -negative patients, G-749 induced strong cell death in comparison with quizartinib and midostaurin. In mice (10 mg/kg p.o.) the compound had C_{max}, t_{max}, half-life and $AUC_{(0\text{-}24\ h)}$ values of 241 ng/mL, 2 hours, 4.1 hours and 2408 ng.h/mL, respectively. The IC_{50} value for hERG was > 3,000-fold higher than the cellular ED_{50} value. In rats, the compound had an oral bioavailability of approximately 41%.

DISCLOSURES

The authors state no conflicts of interest.

The website for this meeting can be found at http://www.aacr.org/home/scientists/meetings—workshops/molecular-targets-and-cancer-therapeutics.aspx.