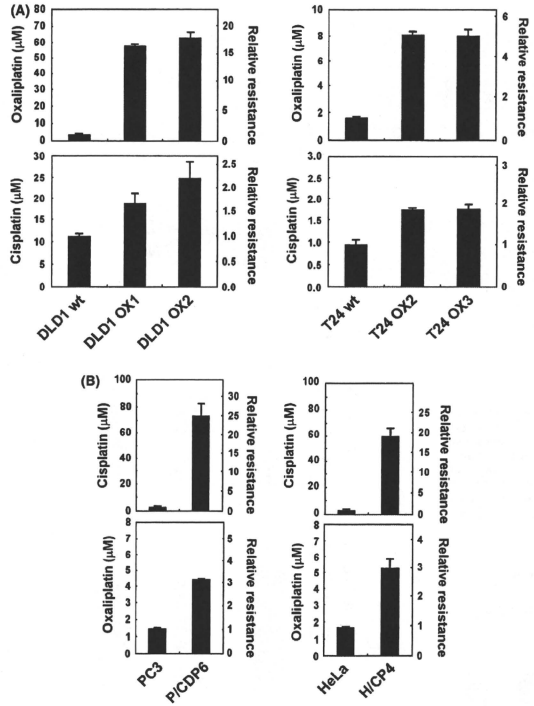

Fig. 1. Establishment of oxaliplatin-resistant colon cancer cells. (A) DLD1 wild-type (wt), DLD1 OX1, and DLD1 OX2 cells (1.0×10^3) were seeded in 96-well plates. The following day, the indicated concentrations of oxaliplatin or cisplatin were applied. After 72 h, cell survival was analyzed using a water-soluble tetrazolium salt-8 assay. Cell survival in the absence of drugs corresponded to 100%. The longitudinal axis indicates the IC50 of each drug. All values represent the mean of at least three independent experiments. (B) Calculation of IC50 for oxaliplatin or cisplatin using cisplatin-resistant cells (P/CDP6 and H/CP4) was carried out as described in (A). The longitudinal axis indicates the IC50 for each drug. All values represent the mean of at least three independent experiments.

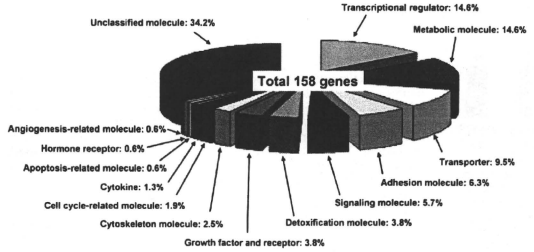

Fig. 2. Classification of genes upregulated in oxaliplatin-resistant cells. The results of DNA microarray analysis showed that 158 gene expressions were commonly increased in DLD1 OX1 and DLD1 OX2 compared with their DLD1 parents. These genes are categorized into 14 groups by their molecular function. Percentages indicate the proportions of genes.

that are expected to be consistently and commonly expressed in multiple pairs of drug-sensitive and -resistant cell lines. With this in mind, we established oxaliplatin-resistant cell lines from two different cancer cell lines and searched for genes relevant to oxaliplatin resistance using a cDNA microarray approach. Gene expression profiles were investigated using DLD1 OX1 and DLD1 OX2 cells (Table S1). One hundred and fifty-eight genes were identified as being upregulated more than 3.0-fold in both DLD1 OX1 and DLD1 OX2 cells. The majority of these genes are transcriptional regulators, metabolic molecules, or transporters. However, approximately 34% were unclassified, suggesting that the molecular mechanisms underlying oxaliplatin resistance are complicated and pleiotropic.

In the present study, we showed that the upregulation of NFIB is an important mechanism involved in oxaliplatin sensitivity. We have not established the revertant clones. Further

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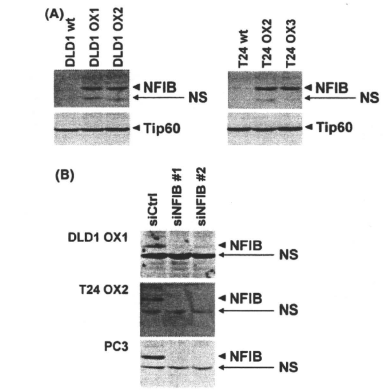

Fig. 3. Overexpression of nuclear factor I/B (NFIB) in oxaliplatin-resistant cells. (A) Nuclear extracts (100 μg) from the indicated cell lines were subjected to SDS-PAGE, and Western blot analysis was carried out using the anti-NFIB and anti-Tip60 antibodies. The anti-Tip60 antibody was used as an internal control. (B) DLD1 OX1, T24 OX2, and PC3 cells were transfected with NFIB siRNA (siNFIB) #1 and #2 and control siRNA (siCtrl). After 48 h, nuclear extracts were prepared and Western blot analysis was carried out using the anti-NFIB antibody. NS, non-specific signal; wt, wild-type.

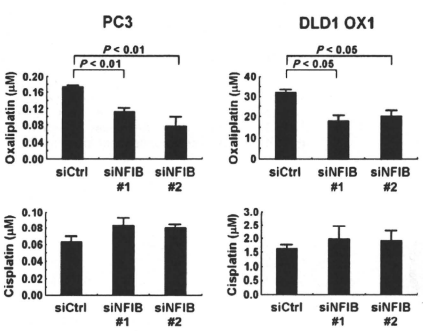

Fig. 4. Sensitization to oxaliplatin by knockdown of nuclear factor I/B (NFIB). Three hundred PC3 or DLD1 OX1 cells transfected with NFIB siRNA (siNFIB) #1 and #2, and control siRNA (siCtrl) were seeded in 35-mm dishes with 2 mL culture medium. The following day, the cells were treated with the indicated concentrations of oxaliplatin or cisplatin. After 7 days, the number of colonies was counted. The colony numbers in the absence of drug corresponded to 100%. Each IC50 was calculated from the concentration–response curve. The longitudinal axis indicates the IC50 for each drug. All values represent the mean of at least three independent experiments.

investigation of NFIB expression in the revertant clones is important to confirm our results. Nuclear factor I/B is a member of the human CCAAT-box binding transcription factor/nuclear factor I (CTF/NFI) family, and was initially shown to promote the transcription and replication of adenovirus type-2.[29] The molecular mechanism of NFIB-dependent transcriptional activation is still unknown, but NFI binding sites have been found in a variety of viral and cellular gene promoters. Platinum antitumor compounds such as cisplatin, carboplatin, and oxaliplatin bind to cellular DNA and form platinum-DNA adducts. Numerous nuclear proteins preferentially bind to cisplatin-DNA adducts, including high-mobility group proteins, YB-1, and TATA-binding protein.[30,31] We

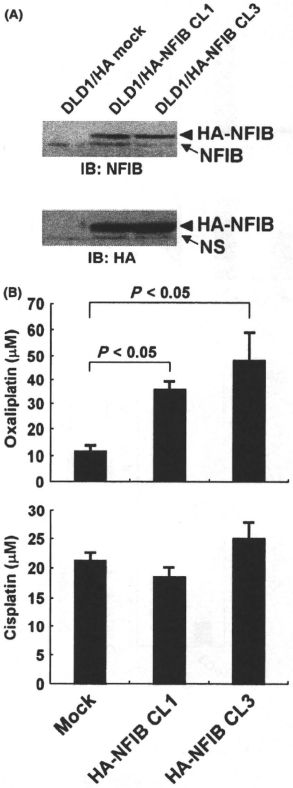

Fig. 5. Oxaliplatin resistance of DLD1 colon cancer cells stably expressing nuclear factor I/B (NFIB). (A) Nuclear extracts (100 μg) and whole cell lysates (50 μg) from the indicated cell lines were subjected to SDS-PAGE, and Western blot analysis was carried out using anti-NFIB and anti-hemagglutinin (HA) antibodies. (B) DLD1 stably expressing HA-NFIB, HA-NFIB CL1 and CL3, as well as mock transfectant (Mock) cells (1.0×10^5) were seeded in 96-well plates. The following day, the indicated concentrations of oxaliplatin or cisplatin were applied. After 72 h, cell survival was analyzed using a water-soluble tetrazolium salt-8 assay. Cell survival in the absence of drugs corresponded to 100%. The longitudinal axis indicates the IC50 for each drug. All values represent the mean of at least three independent experiments.

reported that there are two NFI binding sites in the promoter region of high mobility group protein B1 (HMGB1) and that both nuclear factor 1 C-type isoform 2 (NFIC/CTF2) and HMGB1 are overexpressed in cisplatin-resistant cells.[32] This NFIC/CTF2 is now categorized as NFIC, different from NFIB. However, it is noteworthy that two different NFI families are overexpressed in different platinum-containing drug-resistant cells. Conformational differences between cisplatin- and oxaliplatin-DNA adducts has been shown,[33] indicating that differential recognition by nuclear proteins may be critical to the differences in efficacy, toxicity, and cellular resistance. Differential expression of the *NFIB* and *NFIC* target genes may also account for the different mechanisms responsible for cisplatin and oxaliplatin resistance. The expression of the *NFIB* gene in human clinical samples may provide a translational tool for oxaliplatin-based chemotherapy.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of 158 genes with fold changes >3.0.

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White Paper

Developments for a growing Japanese patient population: Facilitating new technologies for future health care

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ABSTRACT

Lung cancer, COPD and cardiovascular diseases are highlighted as some of the most common disease that cause mortality, and for that reason are the most active areas for drug development. This perspective paper overviews the urgent need to develop a health care system for a rapidly growing patient population in Japan, including forthcoming demands on clinical care, expecting outcomes, and economics. There is an increasing requirement to build on the strengths of the current health care system, thereby delivering urgent solutions for the future. There is also a declaration from the Ministry of Health, Labour and Welfare (MHLW), to develop new biomarker diagnostics, which is intended for patient stratification, aiding in diagnostic phenotype selection for responders to drug treatment of Japanese patients.

This perspective was written by the panel in order to introduce novel technologies and diagnostic capabilities with successful implementation. The next generation of personalized drugs for targeted and stratified patient treatment will soon be available in major disease areas such as, lifestyle-related cancers, especially lung cancers with the highest mortality including a predisposing disorder chronic obstructive pulmonary disease, cardiovascular disease, and other diseases. Mass spectrometric technologies can provide the "phenotypic fingerprint" required for the concept of Personalized Medicine. Mass spectrometry-driven target biomarker diagnoses in combination with high resolution computed tomography can provide a critical pathway initiative facilitated by a fully integrated e-Health infrastructure system. We strongly recommend integrating validated biomarkers based on clinical proteomics, and medical imaging with clinical care strategy supported by e-Health model. This will help to create personalized treatment paradigms and to reduce mortality and healthcare costs of chronic and co-morbid diseases in the elderly population of Japan.

Contents

1. Japanese health care

The elderly population (above 65 years of age) in Japan is currently 21% (27.4 million) reaching 25.2% by 2020, and 40% by 2055. The estimated doubling time (from 7% to 14%) of the 65 and older population is calculated to be 26 years, compared to a 75-year doubling time in the US, and presents a unique demographic shift toward an aging population. In comparison to other countries, Japan is unique, with an overwhelming number of elderly population, which is close to 40%, as predicted by 2050. This should be compared to the US, where the prediction of the transition of elderly population is at about the half, 20%. These data on long-term transition of elderly population rate in eight countries have been published recently (2009) by the National Institute of Population and Social Security Research. There is a general global trend of an increase of the elderly population. The comparative predictions of the elderly age group for Japan in-between 2010 and four decades ahead (until 2050) is an increase of 60%, as illustrated in Fig. 1. The data also predict that with a perspective of a century the increase of the elderly population in Japanese society is expected to grow with 100%, which is remarkable. As a general global trend, the elderly population is growing with varying speed in the respective countries.

Currently, Japan has a low cost functional system with the current healthcare costs accounting for 8.5% of GDP (or nearly 2100 US$ per capita), including 85% contribution by public

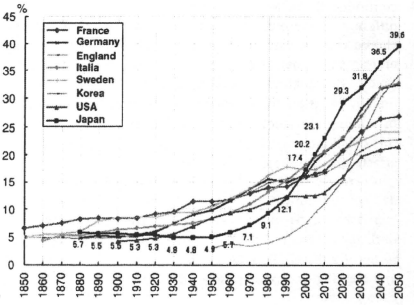

Fig. 1 – A prominent demographic shift toward the elderly population in Japan. The ordinate indicates the percent of the 65 and older population.

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funds. However, the change in elderly population demographics will create increased demand on the healthcare system, thereby requiring for innovations in healthcare and treatment.

With the elder population expanding, the lifestyle-related cancer rates will inevitably increase. Smoking-related lung cancer's mortality has already been the highest among all cancers in both Japan and United States. Chronic obstructive pulmonary disease (COPD) is another tobacco- or air pollution-related disease, and can predispose patients to lung cancers. Japan is the world's largest market for tobacco products (smoking rate at 29%) and has COPD prevalence estimates similar to western countries with over 5 million COPD patients [1]. The cost impacts of COPD in Japan have been huge and estimated at 805.5 billion ¥ (6.8 billion US$) per year; 645.1 billion ¥ (5.5 billion US$) in direct costs and 160.4 billion ¥ (1.4 billion US$) in indirect costs (direct and indirect costs are split as 80% to 20%). COPD is a preventable disease with cessation of smoking, which has widely been promoted to reduce the prevalence of the disease; it will take longer time for the promotion to become indeed effective. Given its complexity and the long term effects of smoking, COPD requires early detection and therapeutic evaluation with comprehensive multi-modalities, and further detection and management of co-morbidities (such as lung cancer and heart disease) that modify outcomes of the primary disease [2,3]. Lung cancer is a "multifactorial" disease, *i.e.*, many factors work together to cause the disease. Most lung cancer patients actually have COPD with progressed emphysema and infectious disease agents such as *Chlamydia pneumoniae*, human papilloma virus (HPV) and measles [4–6]. Possibly, these factors in combination with certain genetic changes may be the initial cause of lung cancer. Researchers are now beginning to isolate some of the genomic factors that are associated with an increased risk of lung cancer. Paradoxically, the incidences of chronic heart disease (CHD) and atherosclerosis in Japan have been in decline. This may be attributed to lower serum cholesterol, declining rates of smoking and declining trends in blood pressure [7]. However, CHD continues to be a major cause of death in smokers with different co-morbidities. A number of studies in Japan have shown that smoking increases the risk of premature death among both men and women. In conclusion, it is likely that environmental factors, in addition to organic cooking (mostly in Asian countries), as well as occupational reasons in addition to smoking are risk factors that contribute to the development of cancer and CHD. Thus, the smoking effects are the major factors for these diseases.

Currently there are 109 unique protein biomarkers used daily in the clinic [8,9]. There are limited studies available for biomarker of diseases except some of the cancer biomarkers until today. Examples like; human epidermal growth factor receptor 2 (HER2), KL6, SA100, prostate specific antigen (PSA) and creatine phosphokinase (CPK) are some markers that are used globally today.

Within lung cancer, there have been reports on early indication of somatic mutation appearances within the EGF receptor. The increased mutation frequency was observed especially in Japan and Asia [10]. The Japanese lung cancer patients showed to have a certain percentage of non-responders, the reason for this was not well understood at the time. At a later time point, when the number of Gefiinib (IRESSA) treated patients increased to tens of thousands, the mutations within the EGF-receptor was discovered at high frequencies [8,11–13]. Later, these somatic mutations were shown to have a direct link to the specific inhibitory effects of IRESSA. Today, an EGFR-mutation assay outcome will guide the clinicians in Japan, to what treatment and medication to use for these patients. Epidermal growth factor receptor is associated to resistance to chemotherapy as has been the case with radiation therapy. The restricted treatment efficacy opened up for novel drugs such as Gefitinib and Erlotinib, developed as specific EGFR-tyrosine kinase inhibitors (TKI), with good efficacy and less side effects. A recent study also presented the situation in Europe [14].

It is evident in Asian populations that the majority of the non-small cell lung cancer (NSCLC) patients with activated mutations achieved a durable and effective response to EGFR TKI-treatment, such as Gefitinib [15,16].

The somatic mutation assay test has now been put into place in Japan, and is used routinely to identify the various lung cancer phenotypes. In addition, a large case–control study was conducted in Japan, involving 52 clinical centers throughout Japan. This epidemiological study was also directed towards biomarker discovery and probably makes it the biggest clinical Biomarker Discovery study undertaken within the industry [17,18].

The future medical treatment of patients is expected to have an increased need to combine diagnosis such as imaging and biomarkers with selection of drug prescriptions for patients. These expected developments are currently being assessed by the FDA and NIH, in collaborative programs and studies with the Pharma industry.

To address the future challenges that Japanese society is facing, novel technologies and diagnostic capabilities must be developed and implemented throughout the coming decade. It is steadily advancing to discover novel target biomarkers that are directly related to pathophysiology and etiology, and to develop diagnostic strategies with those markers. Imaging modalities including high resolution computer tomography (HRCT), magnetic resonance imaging (MRI) and positron emission tomography (PET) have been commonly used for diagnosis in average Japanese hospitals and even in health-care clinics. However, these proteomic and imaging methods are now being used separately and provide mutually independent information. COPD involves multiple compartments in the lung, such as the airways, parenchyma, as well as vessels, and causes remodelling and destruction, which can vary among individuals. The genetic basis for such a difference in susceptibility and disease presentation is currently being elucidated and potential genes implicated are currently validated [19]. CT offers a non-invasive approach to image COPD disease changes [20] at a spatial resolution of 0.5 mm in X and Y directions and 1 mm in Z direction which helps in accurately resolving changes in airways of around 2 mm [21]. However, additional novel techniques like optical coherence tomography (OCT) can provide a spatial resolution of around 3 to 16 μm and an ability to image at a surface depth

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of 3 mm [22]. Currently, new developments are progressing where the combination of HRCT imaging and target biomarker expression analysis may assess a correlation between histopathological changes and biomarker levels. This interdisciplinary approach will help to identify disease at an early stage and the degree of progression, and thus to improve an individual patient's outcome. Upon drug treatment, changes in the CT-image and biomarker assay read-outs will indicate outcomes for the patient. The ultimate goal would be to monitor the treatment response in clinical and functional variables that show good correlation to CT measurements with HRCT, with less variability than the placebo group.

Another important consideration, which remains as a top priority for future Personalized Medicine developments, is the drugs with low frequency of adverse events. Patient safety, which relates to the minimization of side effects, is crucial in order to limit the suffering of patients, as an effect of drug use.

Japan has declared a pricing strategy that includes request for new biomarker diagnostics that can be used for patient stratification, with phenotype selection for responders to drug treatment [23]. In this declaration, pharmaco-genomic, and proteomic technologies are promoted in the discovery and development of drug related biomarkers by the drug pricing committee within the Ministry of Health, Labour and Welfare (MHLW), (http://www.mhlw.go.jp/shingi/2009/07/dl/s0715-9a.pdf).

The pricing strategy will be used in order to promote safe and efficient approved drugs for the treatment of Japanese patients.

2. Protein biomarker diagnosis

Detection of new biomarkers of emphysema and inflammatory reaction in the lung and heart can aid in early identification of disease and in monitoring the effect of therapeutic agents on disease progression. There is currently much research activity in this area but no consensus regarding which bio-molecules are most useful for the identification of COPD progression or for predicting clinical outcomes.

It is expected that multiplexed biomarker assay platforms will play an important clinical role as becoming a complement to traditional immuno-assays for future molecular diagnostics. An early evidence of the progress developments is that recently, the interagency group of the National Cancer Institute and the Food and Drug Administration (NCI-FDA) presented the validation of protein based multiplex assay [24]. In addition, they reported on Multiplexed Biomarker Assay Platform developments where the NCI-FDA Oncology Task Force, members of the Clinical Proteomic Technology Assessment for Cancer program, are evaluating both antibody based multiplexing as well as mass spectrometry based MRM assays [25].

Multiplexed biomarker assay platforms are expected to be the key platforms that will help improve the clinical health care, and targeted medication in the future. The mass spectrometry based MRM assay panels would be using the same SRM/MRM principles as for drug and metabolite monitoring. These quantitative multiple reaction monitoring (MRM) methods have been in use for more than a decade in the development of new medicines, and this has been in close collaboration with the FDA.

Currently the available triple-quadrupole mass spectrometers have both improves sensitivity, mass accuracy as well as scan speed that is in line with the multiplex measurement principle.

3. Multiplexed biomarker assay platforms

Mass spectrometry-based selective reaction monitoring is rapidly developing with an expectation to become a preferred technology for the development of quantitative protein or peptide assays with high sensitivity and selectivity for clinical research [26,27]. Its sequential application to multiple targets at once MRM delivers high-throughput, and when taken together, these parameters provide a breakthrough quantification methodology. This technology allows absolute biomarker quantification in very small amounts of bio-fluid samples, allowing multiplexed read-outs of disease panels.

Assay formats with multiple hundreds of proteins/assay have recently been presented [28,29]. It is expected that clinical assay panels with 100 proteins/assay, screening both blood samples and tissues will be standard in laboratories around the world in the near future. The MRM assay format measures specific target proteins by monitoring *proteotypic* (unique to the target protein) peptide sequences. The technology is fully quantitative when isotopically labeled internal standards are included in the assay. No immuno-reagents are required for MRM assays in principle but immuno-precipitation or other affinity enrichment techniques may be used in sample preparation to enhance the sensitivity of the assay. The MRM technology is performed on triple quadrupole mass spectrometers and provides precise quantification and broad dynamic range, even within highly complex sample matrices. The multiplex MRM assays allow high density data generation in clinical diagnosis, where it is envisioned that multiple MRM-panels can be run simultaneously. This would provide a whole new setting, whereby the health care system would perform future patient diagnosis. In fact, immunoassay platforms like ELISA, with extensive robotics and automation would face major difficulties fulfilling these performances. Even with current synthesis technologies of isotope labeled internal standards, it makes MRM-assay costs highly competitive in comparison to current pricing in the clinical hospitals running clinical assays.

In addition, when mass spectrometry is coupled with a front-end sample introduction system such as nano-flow liquid chromatography, the limit of quantification/detection of target peptides/proteins and biomarkers may reach low attomole levels. The utilization of this technology makes analysis of clinical "fingerprint" target biomarkers in common body fluids not only possible, but also an attainable goal for the future. Recent promising efforts to combine MRM with sampling at histological levels [30], will facilitate finding the body fluid targets of which levels correlate with those at disease foci, serving as a diagnostic strategy combined with HRCT. MRM delivers a unique signal that can be detected and quantified in the midst of a very complicated biological matrix. The mass spectrometry spectra plots are simple, usually containing only a single peak for each MRM. This

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characteristic makes the assay especially suitable for sensitive and specific quantitation [31].

The latest developments within the MRM technology are providing high density assay panels with high sensitivities, allowing low abundant level proteins to be quantified, even down to copy numbers as low as 40 copies/cell [28].

In comparison with ELISA immunoassays, recently MRM panels were presented in blood plasma with linear operational performance down to low pg/ml [24].

Imaging using CT, MRI, ultrasound, molecular imaging is commonly used in clinical practice and also in therapeutic trails. They help in non-invasively quantifying regional disease, which is critical for validating clinical proteomic biomarkers elucidated from different tissue compartments.

Computed tomography (CT) is considered a novel modality to estimate the key pathological changes in COPD patients, namely emphysema and airway remodeling. Japan is in the forefront of CT technology and has access to the advanced scanner hardware and required expertise in radiology and informatics. CT offers a non-invasive approach to image COPD disease changes [20] at a spatial resolution of 0.5 mm in X and Y directions and 1 mm in the Z direction which helps in accurately resolving changes in airways of around 2 mm [21]. By accurate quantification of the disease pathology CT allows phenotyping (or patient stratification) for evaluating novel treatments or determining prognoses. Several academic groups and large chest radiology consortia like the Fleischner Society (www.fleischner.org/) continue to bring novel insights into the application of CT technology and standardization of the method for evaluating COPD and the Big3 diseases, i.e., "lung cancer", "COPD" and "atherosclerosis". By using automated CAD tools in batch mode, image analysis and quantification can be done in high-throughput allowing application to large-scale databases.

However, in addition novel techniques like optical coherence tomography (OCT) can provide a spatial resolution of around 3 to 16 μm and an ability to image at a surface depth of 3 mm [22]. MRI imaging using hyperpolarized gases like helium, xenon and fluoride and molecular imaging with PET offer exciting insights into functional status.

Lung cancer imaging using CT together with PET for imaging the volume and functional status of lung nodules is widely used in clinical setting. These tools are used in screening of large cohorts of smokers and currently the benefits of screening are being evaluated. Together with tumor based proteomic biomarkers, imaging offers strong model to related function, structure and disease stage with proteomic finger print, thereby supporting the validation of clinical proteomic endpoints in smoking related lung diseases. In addition, a large part of the lung cancer patients with diagnosed tumors, also show radiological evidence of emphysema and airway disease [32,33].

Critical path initiative (CPI) is the US national strategy for transforming the way FDA-regulated products are developed, evaluated, manufactured, and used with a focus on accelerating the development of safe and efficacious novel treatments (http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/default.htm). CPI has initiated several opportunities centered around the development and validation of novel biomarkers for smoking-related diseases including soluble biomarkers, patient-reported outcomes and imaging. NHLBI/NIH has initiated a program for Sub Populations and InteRmediate Outcome Measures In COPD (SPIROMICS), which focuses on combining biomarkers and imaging endpoints for outcome assessment in COPD patients.

In *eHealth developments*, the exchange of health information electronically between physicians, hospitals, health plans, and patients has increased substantially in the last year and is reducing the cost of care and positively impacting physicians, according to a new survey released by the non-profit eHealth Initiative (eHI) today. "Migrating Toward Meaningful Use: The State of Health Information Exchange," a report based on eHI's *Sixth Annual Survey of Health Information Exchange*, presents a very clear benefit to the health care system. Responses from operational initiatives demonstrate an increasingly positive impact on the efficiency of care while showing a return on investment.

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ORIGINAL ARTICLE

Phase I study of LY2181308, an antisense oligonucleotide against survivin, in patients with advanced solid tumors

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Abstract
Purpose LY2181308 is an antisense oligonucleotide that complementarily binds to survivin mRNA and inhibits its expression in tumor tissue. This phase I dose escalation study evaluated the tolerability, pharmacokinetics, and anticancer activity of LY2181308 in Japanese.
Methods Patients with solid tumors refractory to standard therapy received LY2181308 (400, 600, or 750 mg) as a 3-h intravenous infusion for 3 consecutive days and thereafter once a week.
Results LY2181308 was administered to 14 patients, aged 44–73 (median 60) years. Flu-like syndrome, prolonged prothrombin time-international normalized ratio (PT-INR), thrombocytopenia, and fatigue were common reversible grade 1/2 toxicities. The dose-limiting toxicity was reversible grade 3 elevation of ALT/AST/γ-GTP in 1 patient treated at the 750-mg dose. Pharmacokinetic analysis showed a long terminal half-life of 21 days and an extensive tissue distribution of LY2181308. In 12 evaluable patients, one patient had stable disease, while the remaining 11 patients had progressive disease.
Conclusions LY2181308 monotherapy is well tolerated up to 750 mg with a manageable toxicity, the pharmacokinetic profile warrants further evaluation of LY2181308 in combination with cytotoxic agents or radiotherapy.

Keywords Antisense oligonucleotide · Pharmacokinetics · Phase I · Survivin

Introduction

Survivin, a member of the inhibitor of apoptosis family of proteins (IAP), regulates apoptosis and promotes cell proliferation [1]. Survivin is highly expressed during fetal development and rarely detectable in normal adult tissues [1]. However, overexpression of survivin has been reported in the vast majority of solid tumors and leukemias [2, 3].

LY2181308 is a novel second-generation antisense oligonucleotide (ASO) designed to complementarily bind to human survivin mRNA, inhibit the gene/protein expression, and consequently restore the normal apoptotic pathway in cancer cells. The antitumor activity of LY2181308 is correlated with inhibition of survivin [4, 5]. Furthermore, LY2181308 potently inhibited the expression of survivin mRNA and protein in human tumor cell lines [6]. These results justified its evaluation in clinical studies. Recently, a first-in-human dose study established the tolerability of LY2181308 at 750 mg [7]. ASOs are known to accumulate in the liver, where they can cause off-target hepatic toxicities [8].

In Japanese patients, genetic variations that influence metabolism and safety of anticancer drugs, such as UDP-glucuronosyltransferase (UGT) 1A1 and multidrug resistance protein (MRP) 1, have been identified [9]. Also, there are genetic variations that can predispose Japanese patients

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to an altered response to inflammation as highlighted by polymorphisms of the TNF gene [10]. Because ASO administration is associated with complement activation leading to subsequent inflammatory reactions, including TNF release [11], Japanese patients may be more susceptible to ASO administration and its associated off-target inflammation. Hence, the objective of this study is to determine the tolerability, pharmacokinetics, and anticancer activity of LY2181308 in Japanese patients with advanced solid tumors.

Materials and methods

Patient eligibility

Patients with malignant solid tumors were eligible after failing standard therapies or if there was no approved treatment available. Eligibility criteria included: age 20–75 years; Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1; adequate bone marrow, hepatic, renal, and coagulative function (absolute neutrophil count $\geq$1,500/μl, platelet count $\geq$100,000/μl, hemoglobin level 9.0 g/dl, total bilirubin $\leq$ upper limit of normal range [ULN], aspartate aminotransferase [AST] or alanine aminotransferase [ALT] $\leq$ 2.5 times ULN, estimated creatinine clearance $\geq$ 50 ml/min, normal prothrombin time-international normalized ratio [PT-INR, 0.8–1.2], and normal activated partial thromboplastin time [APTT, 23-40 s]); discontinuation of prior anticancer therapy 28 days before enrollment to this study or conclusion of palliative radiotherapy 14 days prior to starting on study; written informed consent. Exclusion criteria included the following: diagnosed pregnancy or ongoing lactation; symptomatic brain metastasis; active hepatitis B, C, or human immunodeficiency virus (HIV); and concomitant use of any anticoagulant drugs. The protocol was approved by the Institutional Review Board. The study was consistent with Good Clinical Practice and all applicable laws and regulations in Japan.

Drug administration and dose escalation procedure

LY2181308 (Eli Lilly and Company, IN, USA) was administered via intravenous infusion over 3 h on day 1–3 of the initial cycle (cycle 1 [day 1–7]) as a loading dose and then once a week as maintenance dose (cycle 2 [day 8–28] and onwards [28 days/cycle]). The content of each vial containing 100 mg in 4 ml of buffer was added to 500 ml of saline. The starting dose was 400 mg, with subsequent dose escalations to 600 and 750 mg in a classical 3 + 3 design. Dose-limiting toxicities (DLTs) were evaluated at each dose level (400:600:750 mg = 3:3:6 patients). The maximum tolerated dose (MTD) was defined as the highest dose level at which no or one patient experienced a DLT.

DLT was defined as any event that met at least one of the following criteria: (1) febrile neutropenia or grade 4 neutropenia persisting more than 4 days; (2) grade 4 decreased hemoglobin or thrombocytopenia, or thrombocytopenia requiring blood transfusion; (3) grade 3 APTT prolongation observed at the end of the study drug administration and persisting $\geq$48 h after administration; (4) non-hematological toxicities of grade 3 or higher (nausea, vomiting, anorexia, fatigue, constipation, diarrhea, and abnormal electrolytes were not considered DLTs if controllable with appropriate treatment); (5) discontinuation/postponement of study drug administration due to a toxicity other than those mentioned above and the total amount of administered study drug <80% of the total amount scheduled for the first 28 days; and (6) other toxicities that were judged as DLTs by the investigator.

Treatment assessment

Tolerability was evaluated in all patients for toxicities, clinical laboratory tests, coagulants, and vital signs. Toxicities were graded according to the Common Terminology Criteria for Adverse Events version 3.0 [12].

Tumor responses were assessed in patients who had measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) guideline version 1.0 [13].

Pharmacokinetics

Pharmacokinetic evaluation was performed in all patients during cycles 1 and 2. The concentration of LY2181308 in plasma was measured by enzyme-linked immunosorbent assay (ELISA) using antidigoxigenin-AP (Roche) to capture LY2181308. The minimum quantifiable concentration was 1.875 ng/ml in plasma. Non-compartmental pharmacokinetic parameters were calculated. Population pharmacokinetic analysis was also conducted in a model-dependent manner using a NON-linear Mixed Effect Model (NONMEM) program version VI.

Results

Patient characteristics

Fourteen patients in this study had the typical characteristics in a phase I study (Table 1). The most common histology was lung cancer (six patients), followed by pancreatic cancer (four patients). Approximately, half of the patients had more than four prior chemotherapies. A total of 31

Table 1 Patient characteristics

Variable	400 mg ($n = 4$)	600 mg ($n = 4$)	750 mg ($n = 6$)	Total ($n = 14$)
Sex				
Male	3	4	4	11
Female	1	0	2	3
Age, years				
Median	58.5	63.5	59.0	60.0
Range	44–73	60–69	49–66	44–73
Primary tumor type				
Lung	1	1	4	6
Pancreas	2	2	0	4
Intrahepatic cholangiocarcinoma	0	1	0	1
Uterus	1	0	0	1
Esophagus	0	0	1	1
Breast	0	0	1	1
ECOG PS				
0	1	1	6	8
1	3	3	0	6
Prior therapy				
Cancer-related surgery	3	1	4	8
Radiotherapy	0	1	5	6
Chemotherapy	4	4	6	14
≥4 prior chemotherapy regimens	1	2	3	6

ECOG PS eastern cooperative oncology group performance status

cycles were administered; the median number of cycles administered per patient was 2 (range 1–4). The maximum treatment period was 93 days.

Safety and tolerability

DLT was evaluated in 12 of the 14 patients. At the 750-mg dose, one patient with small cell lung cancer and a PS of 0 experienced DLTs of grade 3 elevation including AST, ALT, and γ-GTP on day 8. These events were asymptomatic and resolved without medication. Another patient at the 600-mg dose experienced a grade 3 total bilirubin elevation, which was not considered a DLT but rather a result of tumor progression. Hence, the MTD was established at the 750-mg dose level.

In addition to these toxicities, we observed flu-like symptoms (fever, chills, and hyperhidrosis), prolonged PT-INR, thrombocytopenia, and fatigue as grades 1/2 (Table 2). Almost all flu-like symptoms were observed during the loading dose of day 1–3. They were manageable with oral antipyretic such as acetaminophen. Prolonged PT-INR and thrombocytopenia were also grade 1/2, and there was no medical treatment required such as blood transfusions. Grade 1 fatigue occurred in eight patients. Grade 3 lymphocytopenia and anemia were only observed in one patient treated at the 400-mg dose; they were not considered drug related. Concentrations of complement fragments Bb and C3a were elevated on day 3 and returned to baseline after day 8 (Fig. 1).

Anticancer activity

Twelve of the fourteen patients met RECIST guideline for antitumor response assessment. At the 600-mg dose, 1 patient with intrahepatic cholangiocarcinoma had stable disease, but the remaining 11 patients had progressive disease.

Pharmacokinetics

Pharmacokinetic analysis was performed in all 14 patients (Fig. 2). The interindividual coefficient of variation (CV%) of AUC for LY2181308 was moderate. Pharmacokinetic parameters on day 3 were similar to those on day 1, suggesting no accumulation of study drug in plasma. The multiphasic disposition pharmacokinetic profile of LY2181308 was adequately described by a 4-compartment model with elimination from the peripheral compartment. The mean terminal $t_{1/2}$, distribution clearance (CL), elimination CL, and V_{ss} estimated by the model were 21 days, 2.0 l/h,

Table 2 Drug-related toxicities at selected dose levels

	400 mg ($n = 4$)		600 mg ($n = 4$)		750 mg ($n = 6$)		All ($n = 14$)			
Grade	1–2	≥3	1–2	≥3	1–2	≥3	1	2	≥3	All
Hematological toxicities										
Decreased platelet count	3	0	3	0	4	0	7	3	0	10
Decreased lymphocyte count	0	1	1	0	2	0	2	1	1	4
Decreased Hb	0	1	2	0	0	0	1	1	1	3
Decreased WBC	0	0	1	0	2	0	3	0	0	3
Non-hematological toxicities										
Increased PT-INR	3	0	3	0	5	0	11	0	0	11
Hyperhidrosis	2	0	3	0	6	0	11	0	0	11
Pyrexia	2	0	3	0	6	0	8	3	0	11
Fatigue	3	0	1	0	4	0	8	0	0	8
Increased CRP	2	0	4	0	2	0	8	0	0	8
Prolonged prothrombin time	1	0	1	0	5	0	7	0	0	7
Increased β-2 microglobulin urine	1	0	4	0	2	0	7	0	0	7
Anorexia	2	0	0	0	3	0	4	1	0	5
Chills	1	0	1	0	3	0	5	0	0	5
Diarrhea	2	0	1	0	2	0	5	0	0	5
Increased ALT	1	0	1	0	2	1[a]	3	1	1	5
Decreased blood albumin	2	0	0	0	2	0	4	0	0	4
Headache	1	0	0	0	3	0	4	0	0	4
Injection site pain	2	0	1	0	1	0	4	0	0	4
Nausea	0	0	1	0	3	0	3	1	0	4
Prolonged APTT	1	0	0	0	3	0	4	0	0	4
Increased AST	1	0	1	0	1	1[a]	3	0	1	4
Flushing	1	0	1	0	1	0	3	0	0	3
Hypothermia	1	0	0	0	2	0	0	3	0	3
Increased ALP	0	0	0	0	3	0	2	1	0	3
Increased blood triglycerides	0	0	0	0	3	0	2	1	0	3
Increased γ-GTP	0	0	0	0	1	2[a]	1	0	2	3

ALP alkaline phosphatase, *ALT* alanine aminotransferase, *APTT* activated partial thromboplastin time, *AST* aspartate aminotransferase, *CRP* c-reactive protein, *γ-GTP* gamma-glutamyl transpeptidase, *Hb* hemoglobin, *PT-INR* prothrombin time-international normalized ratio

[a] Grade 3 elevations in AST/ALT/γ-GTP in one patient were judged as DLTs

28.1 l/h, and 2.05×10^5 l, respectively. Model analysis showed that 84.5% of plasma LY2181308 was distributed to tissue within 8 h after the initiation of administration.

Discussion

Survivin is attracting considerable interest as a potential target for cancer therapy because it is upregulated in most malignancies and may play a role in blocking apoptosis in cancer cells [14]. LY2181308 is the first ASO to successfully inhibit survivin. Overall, LY2181308 was generally well tolerated in patients with advanced solid tumors. MTD was reached at 750 mg, and the 750-mg dose was recommended for further investigations. This study revealed a potential mild hepatotoxicity at the 750-mg dose in contrast to the first-in-human dose study in which this event was only observed at doses of 900 and 1,000 mg [7]. Other clinically important toxicities included flu-like symptoms, elevated PT-INR, thrombocytopenia, and fatigue. The frequency of flu-like symptoms, such as fever, was higher (79%) than that of previous study (32%) [7]. Despite these two main differences, LY2181308 had similar tolerability profile in Japanese as in Caucasian or other races [7]. However, the low-grade toxicity, the reversibility profile, and the medical treatment with antipyretics suggest that these differences between two studies may be marginal or insignificant.

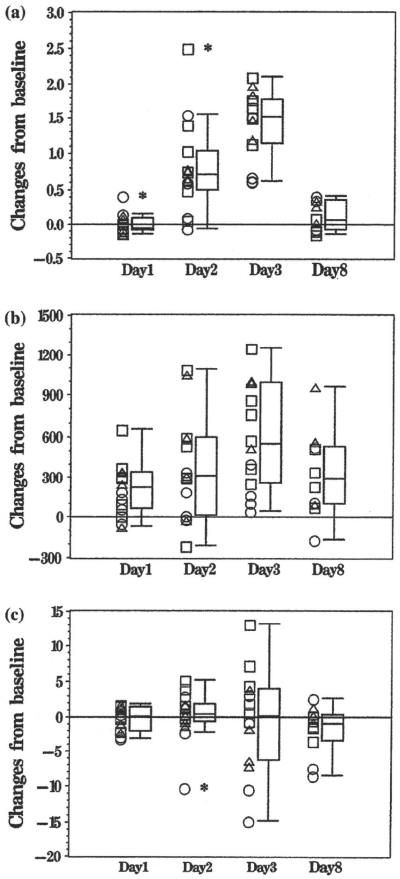

Fig. 1 Change in plasma concentration of complement fragments. Change in plasma concentration of complement fragments from baseline (before administration) to 15 min after the completion of infusion (**a**, Bb; **b** C3a; **c**, C5a). Symbols = *circles* 400 mg/time; *triangles* 600 mg/time; *squares* 750 mg/time. Normal range = Bb, 0.35–0.85 μg/ml; C3a, 305–1,239 ng/ml; C5a, 13.5–58.7 ng/ml

One reason for the induction of flu-like symptoms would be the off-target activation of complement after infusion of ASOs. Increase in levels of complement fragments C3a and Bb on day 3 was comparable to those found in preclinical studies using monkeys [15] and past phase I studies of other ASOs [16]. These complement elevations were acceptable since there was no signs of abnormal immune responses including anaphylaxis. Also, increases in C-reactive protein were equally distributed across all three dose groups, thus suggesting that the complement activation was similar in all patients.

Thrombocytopenia and decreased hemoglobin were observed as grade 1/2. In just one case, grade 3 was observed, but hematologic toxicities were generally mild. The ASO effect on reducing platelet counts may have two potential mechanisms: one is related to the well-known off-target effect of ASOs [8] and the other to the inhibition of survivin. For instance, survivin-depleted mice have reduced hemoglobin, white blood cell, and platelet counts as a result of loss in hematopoietic progenitor cells [17]. Lastly, it should be noted that despite the high accumulation of ASOs in the kidney, no elevation or signs and symptoms related to kidney dysfunction were observed.

The pharmacokinetic profile had a long half-life of 21 days. Consistent with the long terminal $t_{1/2}$, the CL to tissue and elimination CL were low to moderate, and the V_{ss} was large. It is thought that the long terminal $t_{1/2}$ represents plasma-tissue concentration during the tissue elimination phase of LY2181308 once the distribution equilibrium between plasma and tissues has been reached [8]. These results were comparable to the pharmacokinetic results from LY218308 studies in mice and monkeys, showing that rapid tissue distribution (within 24 h) cleared approximately 90% of the drug from plasma. The linearity of LY2182308 was not evaluated in this study due to the small number of patients.

Finally, 12 patients met the RECIST guideline for tumor response assessment. Only one patient had stable disease, while the others had progressive disease. While these data do not suggest any single-agent activity of LY2181308, it should be stressed that this was also not expected. LY2181308 is expected to have activity in conjunction with apoptosis-inducing agents, such as chemotherapy or radiation, and render previously apoptosis-resistant cells sensitive to pro-apoptotic treatments. For instance, paclitaxel or docetaxel resistance has been associated with increased survivin expression [18–20]. Besides, radiotherapy treatment is enhanced after blocking survivin expression in colorectal cancer models [21]. Based on these observations and the favorable toxicity profile of LY2181308, three phase 2 studies are being/have been conducted in conjunction with cytotoxic drugs: (1) combination with cytarabine and idarubicin for acute myeloid leukemia (completed), (2) combination with docetaxel and prednisone for prostate cancer (on-going), and (3) combination with docetaxel for non-small cell lung cancer (ongoing).

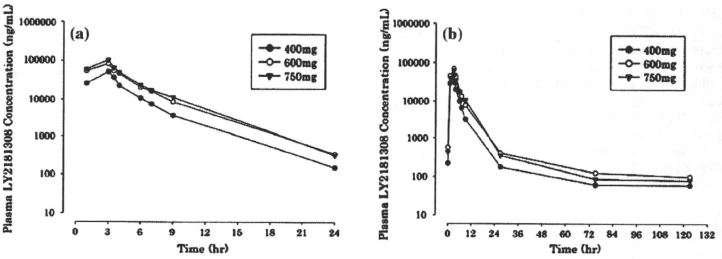

Fig. 2 Mean plasma concentration–time profiles of LY2181308. Mean plasma concentration–time profiles of LY2181308 on day 1 (**a**) and day 3–7 (**b**). C_{max} and AUC at 750 mg were comparable between Japanese and non-Japanese patients [21]

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Conflict of interest Results from this study were presented in part at the 21st (2009) Molecular Targets and Cancer Therapeutics Conference (EORTC-NCI-AACR Symposium; Boston, 15–19 November) [22]. T. Tamura serves as a consultant to Eli Lilly Japan K.K. T. Fujimoto, R. Sekiguchi and K. Uenaka are full-time employees of Eli Lilly Japan K.K. S. Callies is a full-time employee of Eli Lilly and Company. M. Tanioka, H. Nokihara, N. Yamamoto, Y. Yamada, K. Yamada and Y. Goto have no conflicts of interest to disclose.

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Phase I and Pharmacokinetic Study of ABI-007, Albumin-bound Paclitaxel, Administered Every 3 Weeks in Japanese Patients with Solid Tumors

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Objective: ABI-007 is a novel Cremophor® EL-free nanoparticle albumin-bound paclitaxel. This Phase I study was designed to evaluate tolerability and determine recommended dose for Japanese patients when ABI-007 was administered in every-3-week schedule. Pharmacokinetics of paclitaxel was also assessed.
Methods: Patients with advanced solid tumors refractory to standard therapy received a 30 min intravenous infusion of ABI-007 every 3 weeks without pre-medications at 200, 260 or 300 mg/m^2, respectively. Tolerability and recommended dose were determined by the standard '3 + 3' rule.
Results: No dose-limiting toxicity was observed, despite the dose escalation. In another cohort, 260 mg/m^2 was re-evaluated and resulted in no dose-limiting toxicity. Grade 3 or 4 neutropenia was reported for the majority of patients ($n = 8$) but no incidence of febrile neutropenia. Non-hematological toxicities were generally mild except for Grade 3 sensory neuropathy ($n = 3$). Pharmacokinetic study demonstrated the area under the curve of paclitaxel increased with increasing the dosage, and comparable pharmacokinetic parameters to the western population. Partial response was observed in three non-small cell lung cancer patients. Two of whom had received docetaxel-containing chemotherapy prior to the study.
Conclusions: ABI-007 administered in every-3-week schedule was well tolerated up to 300 mg/m^2, and recommended dose was determined at 260 mg/m^2 in consideration of efficacy, toxicities and similarity of pharmacokinetic profile in western studies. Additional studies of single-agent ABI-007 as well as platinum-based combinations, particularly in patients with non-small cell lung cancer, are warranted.

Key words: nanoparticle albumin-bound paclitaxel – ABI-007 – Phase I – pharmacokinetic – Japanese

INTRODUCTION

ABI-007 (Abraxane®; Abraxis Bioscience, Los Angels, CA, USA) is a novel Cremophor® EL (polyoxyethylated castor oil)-free albumin-bound nanoparticle formulation of paclitaxel. This formulation allows for a higher paclitaxel concentration in the suspension, serving to reduce the administration volume and time. No pre-medication to prevent the Cremophor® EL-induced hypersensitivity reaction is needed. In addition, non-polyvinyl chloride infusion system and in-line filtration are not necessarily applied given no leaching of plasticizers (1,2).

In the Phase I study of every-3-week (Q3W) schedule conducted in the USA, the dose of ABI-007 was escalated from 135 to 375 mg/m^2, and maximum tolerated dose (MTD) and recommended dose (RD) were established at 300 mg/m^2. It was exceedingly higher than that of solvent-based paclitaxel (Taxol®; Bristol–Myers Squibb, Princeton, NJ, USA), 175 mg/m^2 (1). Dose-limiting toxicities (DLTs) were keratitis, blurred vision, sensory neuropathy, stomatitis and neutropenia. Maximum concentration (C_{max}) and the area under the curve from time zero to infinity (AUC_{inf}) of paclitaxel increased linearly over the ABI-007 dose range of 135–300 mg/m^2 administered over 30 min. Volume of distribution of ABI-007 is characterized by the larger distribution than solvent-based paclitaxel, indicating extensive extravascular distribution of the drug (3). C_{max} and AUC_{inf} values for individual patients correlated well with toxicities.

In the Phase III pivotal study of 454 patients with metastatic breast cancer, Q3W schedule of ABI-007 260 mg/m^2 produced the superior outcome to the same schedule of solvent-based paclitaxel, 175 mg/m^2: significantly higher response rate and prolonged time to progression [33% vs. 19% ($P < 0.001$) and 23.0 vs. 16.9 weeks ($P = 0.006$), respectively] and significantly lower incidence of Grade 4 neutropenia, despite a 49% higher paclitaxel dose [9% vs. 22% ($P < 0.001$)] (4). The dosage and schedule used in this Phase III study lead to the approved labeling worldwide.

According to the clinical utility and study data reported overseas, ABI-007 seems to be an effective treatment. This Phase I study aimed to evaluate tolerability, DLT and RD in Japanese patients with solid tumors when administered in Q3W schedule. Efficacy, toxicity and pharmacokinetics (PK) were also evaluated as secondary objectives, followed by the evaluation on ethnic difference in PK.

PATIENTS AND METHODS

Patient Eligibility

Patients aged 20–74 years with histologically or cytologically diagnosed malignant solid tumors refractory to standard therapies or for which there was no effective treatment were eligible. They had to have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2, and a life expectancy of ≥60 days. Eligibility criteria also included adequate renal, liver and bone marrow function, defined as serum creatinine (Cr) ≤1.5 mg/dl, serum total bilirubin (TB) ≤1.5 mg/dl, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) <100 IU/l, respectively, serum albumin ≥3.0 g/dl, white blood cell count ≤12 000/mm^3, absolute neutrophil count ≥2000/mm^3, platelets ≥100 000/mm^3 and hemoglobin ≥9.0 g/dl. Patients with prior exposure to taxanes were eligible for the study. Key exclusion criteria included the following: (i) surgery within 4 weeks; (ii) chemotherapy within 3 weeks; (iii) radiotherapy within 3 weeks; (iv) history of radiation to more than 30% of hematopoietic marrow; (v) pre-existing sensory neuropathy ≥Grade 2; (vi) pleural effusion and ascites that required drainage; (vii) brain metastasis showing symptoms or requiring treatment; (viii) hepatitis B or C virus or human immunodeficiency virus infection; (ix) chronic steroid treatment; (x) pregnancy, lactation, suspicion of being pregnant; (xi) serious pre-existing medical conditions such as uncontrolled infections, pulmonary fibrosis, diabetes, severe heart disease and psychogenic disorders.

This study was approved by the Institutional Review Board at the National Cancer Center and conducted according to Japanese Good Clinical Practice guidelines. All patients provided written informed consent prior to study entry.

Study Design and Treatment

This Phase I, open label, dose-finding study was conducted at National Cancer Center and National Cancer Center East.

ABI-007 was supplied by TAIHO Pharmaceutical Co., Ltd, Tokyo Japan. Each vial contained 100 mg of paclitaxel and ~900 mg of frozen-dried Albumin Human (United States Pharmacopeia). The prescribed dose of ABI-007 was prepared in 5 mg (paclitaxel)/ml of physiological saline as a suspension. The drug was administered via 30 min i.v. without pre-medication and in-line filtration.

Evaluated dose levels were 200, 260 or 300 mg/m^2, as shown in Table 1, repeated every 3 weeks. The rationale for selected dose range was the following: the upper level, 300 mg/m^2—MTD determined in a US Phase I study; the middle level, 260 mg/m^2—the approved dose in the US/EU, and the lower level, 200 mg/m^2—one dose level below MTD examined in the foregoing Phase I study. The dose range also factored in PK: linear PK of ABI-007 over the dose range 80–300 mg/m^2 and the same level and activity of CYP2C8 and CYP3A4 between Japanese and Caucasians (5). Dose escalation was capped at 300 mg/m^2. In the event that MTD exceeded the cap, further steps in investigation would be discussed among study sponsor, principal investigator and medical experts.

The dose escalation followed the standard '3 + 3' rule. Three patients were evaluated at the first dose level, and in the absence of DLTs, three additional patients were entered at the next dose level. If one of the three patients encountered a DLT, another cohort was to be added at the same dose level. The MTD was defined as the dose level at which two out of three to six patients experienced DLT. The RD

Table 1. Dose levels

Level	Dose (mg/m^2)	No. of patients entered	No. of courses
1	200	3	9
2	260	6	23
3	300	3	14

was defined as the dose level that is one level below MTD, and consequently, a total of six patients were to be treated at RD to further evaluate the safety profile.

DLTs were pre-defined as any of the following drug-related toxicities that had occurred during the first course: (i) Grade 4 thrombocytopenia; (ii) Grade 3 thrombocytopenia requiring platelet transfusion; (iii) Grade 4 neutropenia over 4 days; (iv) Grade 3 or 4 febrile neutropenia; and (v) Grade 3 or 4 non-hematologic toxicity. Dose was reduced by one level when DLT occurred in the first course, and reduction was allowed when the toxicities corresponding to DLT or Grade 2 neuropathy occurred in the second course or later.

Patient Evaluation

Pre-treatment evaluation included a complete history and physical examination, a complete blood count with differential, serum chemistry profile, urinalysis including pregnancy test, chest X-ray and electrocardiogram. Serum chemistry profile included electrolytes, Cr, urea nitrogen, TB, AST, ALT, lactic dehydrogenase, alkaline phosphatase, total protein, albumin and C-reactive protein. Baseline imaging studies and serum tumor marker levels were obtained at the discretion of treating physician. Toxicity assessment, physical examination and all blood tests except serum tumor markers were repeated on a weekly basis.

Toxicities were graded according to Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. Patients were considered evaluable for toxicity if they received at least one dose of the study drug. Objective response to therapy was assessed every 4–6 weeks according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0 (6).

Blood Sampling and PK Analysis

Whole blood samples of 7 ml each were collected in 6 ml of heparinized tube and 1 ml of K3-EDTA tube to determine the PK of ABI-007 at time points: 0, 0.25, 0.5 (end of infusion), 0.75, 1, 1.5, 2, 4, 10, 24, 48 and 72 h. Heparinized samples were immediately centrifuged at 1000 *g* for 15 min in 4°C and resultant plasma was stored in aliquot, whereas K3-EDTA samples were softly mixed in normal temperature. These samples were stored at less than or equal to −20°C until analyzed. The sample was analyzed for paclitaxel using liquid chromatography/tandem mass spectrometry in Alta Analytical Laboratory (El Dorado Hills, CA, USA). The limit of quantification for paclitaxel in plasma and whole blood was 1.00 and 5.00 ng/ml, respectively, and the range of reliable response in these samples was 1.00–500 and 5.00–5000 ng/ml, respectively.

PK parameters were determined from each patient's whole blood/plasma paclitaxel concentration profile. They were evaluated by non-compartmental analysis using the WinNonlin software package (Ver4.1, Pharsight Corp., CA, USA). The C_{max} of paclitaxel was obtained directly from experimental data. The elimination constant (λz) was obtained by log-linear regression analysis of the terminal phase of the whole blood/plasma concentration vs. time profile. The elimination half-life ($t_{1/2}$) was determined by taking the ratio of natural log of 2 and λz. The AUC_{inf} was estimated by summing the areas from time zero to the last measured concentration–time point (AUC_{0-t}), calculated using the linear-logarithmic trapezoidal method, and the extrapolated area. The dose–area relationship (i.e. total ABI-007 dose divided by AUC_{inf}) was used to determine total body clearance (CL). The volume of distribution (Vz) was determined by taking the ratio between CL and λz.

Table 2. Patient characteristics

Characteristics	No. of patients
Total no. of patients	12
Male/female	10/2
Age (years)	
Median	61
Range	45–69
ECOG performance status	
0	3
1	9
Tumor type	
NSCLC	6
Parotid gland	1
Ovary	1
Bladder	1
Pharyngeal and esophageal	1
Colon	1
Thymoma	1
Prior treatment	
Surgery	9
Radiotherapy	3
Chemotherapy	12
No. of prior chemotherapy	
1	1
2	4
≥3	7
Prior taxane therapy	
Yes	
Solvent-based paclitaxel	1
Docetaxel	5
Solvent-based paclitaxel and docetaxel	2
No	4

ECOG, Eastern Cooperative Oncology Group; NSCLC, non-small cell lung cancer.

Table 3. Hematologic toxicities (all courses)

Dose levels	Level 1 (200 mg/m^2)			Level 2 (260 mg/m^2)			Level 3 (300 mg/m^2)			All		
No. of patients (no. of courses)	$n = 3$ (9)			$n = 6$ (23)			$n = 3$ (14)			$n = 12$ (46)		
CTCAE grade	1–2	3	4	1–2	3	4	1–2	3	4	1–2	3	4
Leucopenia	2	0	0	3	2	0	3	0	0	8	2	0
Neutropenia	1	1	0	1	3	1	1	2	0	2	6	2
Anemia	1	0	0	2	0	0	1	0	0	4	0	0
Thrombocytopenia	0	0	0	1	0	0	1	0	0	2	0	0

CTCAE, Common Terminology Criteria for Adverse Events.

Descriptive statistics were used for baseline characteristics, safety assessment, and PK variables. Regression analysis of individual C_{max}, and AUC_{inf} vs. dose was performed to gain an appreciation of PK linearity. The SAS software package (ver8.2, SAS Institute, Inc., NC, USA) was used for statistical analysis.

RESULTS

Patients and Treatment

Between August 2006 and June 2007, 12 patients were enrolled and treated in this study at two participating centers in Japan. Patient characteristics are summarized in Table 2. Most patients were male (83%) with a median age of 61 (range, 45–69) years and all patients were ECOG PS 0–1. The predominant type of tumor was non-small cell lung cancer (NSCLC). Nine patients had surgery for primary tumors, seven had received more than three prior chemotherapy regimens and eight had received prior taxane-containing chemotherapy.

The patients were treated at the following dose levels: 200 mg/m^2 (Level 1, $n = 3$), 260 mg/m^2 (Level 2, $n = 6$) and 300 mg/m^2 (Level 3, $n = 3$). All were evaluable for safety and PK, and 11 for efficacy (one had no adequate measurable lesions for RECIST criteria).

DLT, Tolerability and RD

No DLTs were observed through the dose escalation to the highest Level 3; therefore, the MTD was not reached methodologically. To decide on the potential RD, study sponsor, medical advisor and principal investigators jointly reviewed the reference data in the foreign studies (1,4,7) and favored 260 mg/m^2 from tolerability and safety perspectives, particularly the development of cumulative neurotoxicity. Additional three patients were then accrued to 260 mg/m^2 cohort to repeat the assessment. None of DLTs being experienced by the additional patients, 260 mg mg/m^2, was established as RD.

Safety

A total of 46 courses of ABI-007 was administered, and the median number of courses administered per patient was 3 (range, 1–11). No acute hypersensitivity reactions were observed during the infusion period. The most common toxicities were neutropenia, leucopenia, lymphopenia, alopecia and sensory neuropathy. The incidences of hematologic toxicities by dose level are shown in Table 3. Grade 3 or 4 neutropenia was often experienced in more than half of patients throughout the study; however, no febrile neutropenia was observed. The median time to onset of Grade 3 or 4 neutropenia was 15.0 (range, 8–34) days, and the median time to recovery to $<$Grade 2 was 6.5 (range, 3–14) days. There were no episodes of $\geq$Grade 2 or greater thrombocytopenia, and anemia was mostly mild. Frequent non-hematological toxicities were sensory neuropathy, alopecia, arthralgia/myalgia and rash (Table 4). The sensory neuropathy was manifested by paresthesia in a symmetric, stocking/glove distribution, and the median time to the first indication or exacerbation from the baseline was 7 days. The severity of non-hematologic toxicities was generally mild except for three cases of Grade 3 sensory neuropathy at Level 2 ($n = 1$) and Level 3 ($n = 2$), which cumulatively exacerbated from Grade 1 observed in the first week of the first course (range, 3–6 days from the administration) to Grade 3 during the third or later course (range, 3–11 courses from the administration). Among the three patients who experienced Grade 3 sensory neuropathy, one patient had received taxane-containing chemotherapy prior to the study. A variety of ocular toxicities including superficial keratopathy reported in the initial Phase I study of USA were not observed in this study. Treatment delay occurred in one patient at each Levels 2 and 3 due to the neurotoxicity, dose reduction occurred in two patients at each Levels 2 and 3 due to the neurotoxicity, and treatment was discontinued in three patients at each Levels 2 and 3, comprising five patients with treatment-related neurotoxicity and one patient with unrelated neutropenia.