individual wells in a volume of 20 μl, and the cells were incubated for 72 h at 37°C in humidified air containing 5% CO_2. MTT reagents (MTT, Sigma) were then added to each well in a volume of 20 μl, and the cells were incubated for 4 h at 37°C in humidified air containing 5% CO_2. Finally, the growth inhibitory effect of each drug was assessed spectrophotometrically.

Drug treatment, RNA isolation and microarray hybridization

To obtain reference profiles representing the drug-induced genomic response, the PC-14 cells were grown on plastic culture dishes until they reached 80% confluency; they were then treated with TZT-1027, D10, VDS, VCR, VBL, TXL and TXT for 6 h at the IC_{50} concentration of each drug determined by MTT assay for 72 h. Cell pellets of the eight samples, including an untreated control, were collected by centrifugation, and the total RNA from each sample was isolated using a single-step guanidium thiocyanate procedure (ISOGEN; Nippon gene).[40] Single-channel labeling ^{32}P nylon membrane-based cDNA microarrays containing 588 genes were used (Atlas® Human Cancer cDNA Expression Array; BD Biosciences Clontech, Palo Alto, CA, USA). Protocols on array printing, labeling and hybridization are available at the BD Biosciences Clontech web site (http://www.bdbiosciences.com/clontech/atlas/index.shtml) The hybridization intensities on X-ray films (Gel Bond®, FMC Bio Products Rockland, ME, USA) were scanned and quantified using a BAS-2000II scanner and Array Gauge software (Fuji Film, Tokyo).

Microarray data analysis

The intensity values of each gene were log-2-transformed and median-normalized using Excel software. The changes in gene expression induced by drug exposure were calculated for each spot by dividing the intensity of the drug exposure samples by that of the untreated samples. The multidimensional scaling analysis, based on a principle component analysis, was performed using SIMCA-P software v10.5 (Umetrics, Sweden). Three-dimensional rendering of the gene profiles was graphed in a manner such that samples with similar expression profiles would lie closer to each other than those with dissimilar profiles. The heat map, which showed the correlation coefficient between each drug reference profile, was performed by *R* (http://cran.r-project.org/).

Functional analysis of identified genes

To analyze the functions of the clustered genes, a gene ontology analysis was performed using the EASE bioinformatics software package (http://apps1.niaid. nih.gov/david/upload.asp).[41,42] This software package was used to rank functional clusters by statistical over-representation of individual genes in specific categories relative to all genes in the same category on the array. The functional clusters used by EASE were derived from the classification systems of Gene Ontology (GO). The *P*-value to rank categories of genes by over-representation was calculated using Jackknife-Fisher exact probabilities. The threshold for selecting categories was a *P*-value of less than 0.01 and a minimum gene count of more than two. *P*-values in gene ontology are not equal to biological significance but are helpful in focusing on the processes most likely to be associated with the biological phenomena associated with aging. We also conducted further online database searches to refine many specific GO annotations.

Real-time RT-PCR

Real-time RT-PCR was performed using a Smart Cycler system (Takara) and a SYBR Green PCR kit. The reaction solution was assembled in a volume of 25 μl comprised of TaqMan™ Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), forward and reverse primers (final concentration, 0.2 μmol/l each) and cDNA mixture ($\approx$2.5 ng) to produce PCR products specific for *GSTP1* and *TIMP3*. The primers and probes were purchased from Sigma-GenoSys (Tokyo, Japan). The conditions for real-time RT-PCR were preheating at 95°C for 10 min, followed by 40 cycles of shuttle heating at 95°C for 15 s and at 60°C for 20 s. A threshold was set in the linear part of the amplification curve, and the number of cycles needed to reach it was calculated for every gene. Relative mRNA levels were determined using the included standard curves for each individual gene and further normalized to the GAPDH mRNA level. Melting curves were used to establish the purity of the amplified band. The sequences of the primers used for RT-PCR were as follows: GSTO1 forward, 5′-AGG TTC TGC CCG TTT GCT GAG AGG and reverse, 5′-CAA GCT TTC TCA TAG GGG TCA TCC G; TIMP3 forward, 5′-TGC TGA CAG GTC GCG TCT ATG ATG G and reverse, 5′-GCG TAG TGT TTG GAC TGG TAG CCA G; GAPDH forward, 5′-TGA AGG TCG GAG TCA ACG GAT TTG GT and reverse, 5′-CAT GTG GGC CAT GAG GTC CAC CAC.

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Duality of Interest

None declared.

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ZD6474 inhibits tumor growth and intraperitoneal dissemination in a highly metastatic orthotopic gastric cancer model

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Angiogenesis inhibitors have been used to treat some cancers, but the therapeutic potential of these agents for gastric cancer has remained unclear. To investigate their therapeutic potential, we examined the effect of ZD6474, an agent that selectively targets vascular endothelial growth factor receptor-2 (VEGFR-2; KDR) tyrosine kinase and epidermal growth factor receptor (EGFR) tyrosine kinase, in a highly metastatic orthotopic model using an undifferentiated gastric cancer cell line, 58As1. ZD6474 (100 mg/kg/day, p.o., 2 weeks) significantly inhibited tumor growth ($p < 0.05$ *vs.* control) and reduced tumor dissemination into the peritoneal cavity ($p < 0.05$ *vs.* control). In addition, to identify putative tumor biomarkers that would reflect the effects of ZD6474 treatment in clinical settings, we examined the gene expression profiles of implanted gastric tumors treated with ZD6474 *in vivo*. Twenty-eight candidate genes were identified, including *IGFBP-3*, *ADM*, *ANGPTL4*, *PLOD2*, *DSIPI*, *NDRG1*, *ENO2*, *HIG2* and *BNIP3L*, which are known to be hypoxia-inducible genes. These genes and gene products may be useful biomarkers for monitoring the effects of ZD6474 treatment. ZD6474 also improved the survival of mice with implanted another undifferentiated gastric cancer cell line, 44As3. In conclusion, our results suggest that ZD6474 may have clinical activity against gastric cancer, particularly undifferentiated gastric cancer with peritoneal dissemination. We also identified putative biomarkers for monitoring the pharmacodynamic effects of ZD6474 by gene expression profiling.

Key words: ZD6474; gastric cancer; intraperitoneal dissemination; VEGF; oligonucleotide microarray

Various anti-cancer agents have been examined for efficacy against gastric cancer over the past two decades, but the median survival time of patients remains around 7 months,[1,2] and the prognosis of gastric cancer patients remains poor. Peritoneal dissemination is common in patients with unresectable gastrointestinal cancer, and many suffer from peritoneal carcinomatosis in the terminal stage. Because undifferentiated gastric cancer is particularly invasive and often accompanied by peritoneal dissemination,[3] a new treatment strategy is needed.

Vascular endothelial growth factor (VEGF) is a key mediator of tumor growth and is known to have multiple functions in angiogenesis, vascular permeability, and the regulation of endothelial cell proliferation and migration.[4–6] VEGF receptors (VEGFR) are activated by ligand stimulation with VEGF and commonly expressed in vascular endothelial cells. VEGFR-2 (KDR/Flk-1) is thought to be important for angiogenesis.[7] Because the VEGF-VEGFR system plays a key role in angiogenesis and tumor growth *in vivo*, the therapeutic potential of many agents targeting this system is being investigated.[8] A recent study has shown that a combination of monoclonal antibody against VEGF and chemotherapy produces a clinically meaningful survival benefit for patients with metastatic colorectal cancer,[5] and these results may lead to changes in the standard treatment for colorectal cancer.[9]

ZD6474 is a novel orally available VEGFR-2 (KDR) tyrosine kinase inhibitor that is also known to selectively target epidermal growth factor receptor (EGFR) tyrosine kinase, both of which are parts of key pathways in tumor growth.[10–13] We demonstrated previously the evidence suggesting that ZD6474 inhibits angiogenesis and tumor growth by targeting EGFR.[14,15]

In our present study, we tested ZD6474 for an inhibitory effect on tumor growth and intraperitoneal dissemination, and for improvement of survival in a newly established, highly metastatic orthotopic gastric tumor model *in vivo*. In addition, we also identified putative biomarkers to monitor the effects of ZD6474 treatment using gene expression profiling.

Material and methods

Reagents

ZD6474 and gefitinib (Iressa®) were provided by AstraZeneca (Cheshire, UK).

Cell cultures

The newly established highly metastatic human signet-ring cell gastric cancer cell lines 58As1 and 44As3 produce large volumes of ascitic fluid and spontaneously metastasize to the peritoneal cavity after orthotopic (gastric wall) implantation.[16,17] 58As1 and 44As3 and human non-small cell lung cancer cell line PC-9 were maintained in RPMI-1640 medium (Sigma, St. Louis, MO) supplemented with 10% heat-inactivated FBS (Gibco BRL, Grand Island, NY). The PC-9 cells were a gift of Tokyo Medical University. Human embryonic kidney cell line 293 (HEK293) was obtained from the American Type Culture Collection (Manassas, VA) and cultured in DMEM supplemented with 10% FBS. Human umbilical vein endothelial cells (HUVEC) were maintained in EBM-2 medium (Clonetics, Walkersville, MD) supplemented with EGM-2 kit (Clonetics), according to the manufacturer's instructions.

In vitro *growth-inhibition assay*

The cell-growth inhibitory effects of ZD6474 and gefitinib were assessed by the thiazole blue tetrazolium bromide (MTT) assay (Sigma). Briefly, 180 μl/well of cell suspension was seeded on to Sumilon® 96-well microculture plates (Sumitomo Bakelite, Akita, Japan) and incubated in 10% FBS-containing medium for 24 hr. The cells were then treated with ZD6474 at various concentrations (4 nM–80 μM) and cultured at 37°C in a humidified atmosphere for 72 hr. After the culture period, 20 μl volume of MTT reagent was added, and the plates were further incubated for 4 hr. After centrifuging the plates, the culture medium was discarded and the wells were filled with dimethyl-sulfoxide. The optical density of the cultures was measured at 562 nm with Delta-soft software on a

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Macintosh computer interfaced to a Bio-Tek Microplate Reader EL-340 (BioMetallics, Princeton, NJ). The experiment was conducted in triplicate.

Immunoblotting

EGFR, phospho-EGFR (specific for Tyr 1068), and anti-rabbit horseradish peroxidase (HRP)-conjugated antibody were purchased from Cell Signalling (Beverly, MA). Cell pellets were lysed in RIPA buffer (Tris-HCl, 50 mM; pH 7.4; NP-40, 1%; Na-deoxycholate, 0.25%; NaCl, 150 mM; EDTA, 1 mM; phenylmethyl-sulfonyl fluoride, 1 mM; aprotinin, leupeptin, pepstatin, 1 mg/ml each; Na_3VO_4, 1 mM; NaF, 1 mM). Cell extracts were electrophoresed on 7.5% (w/v) polyacrylamide gels and transferred to a polyvinylidene di-fluoride membrane (Nihon Millipore, Tokyo, Japan). The membrane was incubated in Tris-buffered saline containing 0.5% Tween 20 with 3% BSA and then reacted with the primary antibodies and the HRP-conjugated secondary antibody for 90 min each. Visualization was achieved with an enhanced chemiluminescent detection reagent (Amersham Bioscience, Buckinghamshire, UK).

RT-PCR

A 5 μg of total RNA from each cultured cell line was converted to cDNA with a GeneAmp® RNA-PCR kit (Applied Biosystems, Foster City, CA). The primers used for the PCR were: human-specific beta-actin, forward: 5′-GGAAATCGTGCGTGACATT-3′ and reverse: 5′-CATCTGCTGGAAGGTGGACAG-3′; mouse-specific beta-actin, forward: 5′-GAAATCGTGCGTGACATCAAA-3′ and reverse: 5′-TCATGGTGCTAGGAGCCA-3′; VEGF-A, forward: 5′-GCCTTG-CCTTGCTGCTCTAC-3′ and reverse: 5′-CA-GGGATTTTCTTGTC-TTGC-3′; VEGF-C, forward: 5′-AAACAAGGAGCTGGATGAA-GAG-3′ and reverse: 5′-CAATATGAAGGGACACAACGAC-3′; VEGFR-1, forward: 5′-TAGCGTCACCAGCAGCGAAAGC-3′ and reverse: 5′-CCTTTCTTTTGGGTCTCTGTGC-3′; VEGFR-2, forward: 5′-CAGACGGAC-AGTGGTATGGTTC-3′ and reverse: 5′-ACCTGCTGGTGGAAAGAACAAC-3′; VEGFR-3, forward: 5′-AGCCATTCATCAACAAGCCT-3′ and reverse: 5′-GGCAACAG-CTGGATGTCATA-3′; IGFBP3, forward: 5′- AATGCTAGTGA-GTCGGAGGAAGAC-3′ and reverse: 5′- GGCGACACTGCTTTT-TCTTATAAA-3′; ADM, forward: 5′- CCTGGGTTCGCTCGCCTT-CCTA-3′ and reverse: 5′- GGCTGGAGCCCCGTGTG- CTTGT-3′.

PCR amplification was carried out for 35 cycles (95°C for 45 sec, 56–62°C for 45 sec, and 72°C for 60 sec) with a final extension step at 72°C for 7 min. The bands were visualized by ethidium bromide staining.

Sequencing

Exons 18–21 of the EGFR cDNA from the tumor cell lines were sequenced, and the cDNAs were amplified using the following primers: forward, 5′-TCCAAACTGCACCTACGGATGC-3′, and reverse, 5′-CATCAACTCCCAAACGGTCACC-3′. The PCR amplification consisted of 25 cycles (95°C for 45 sec, 55°C for 30 sec and 72°C for 60 sec). The sequences of the PCR products were determined using ABI prism 310 (Applied Biosystems). Amplification and sequencing were carried out in duplicate for each tumor cell line. The sequences were compared to the GenBank-archived human sequence of EGFR (accession number: NM_005228.3).

Orthotopic model in vivo

ZD6474 was dissolved in sterile water containing 1% TWEEN 80 (Sigma). Six-week old female BALB/c nude mice were purchased from CLEA Japan Inc. (Tokyo) and maintained under specific pathogen-free conditions. A total of 1×10^6 58As1 cells was inoculated into the gastric wall of each mouse after laparotomy. Three days after the inoculation, the mice were given ZD6474 50 mg/kg/day ($n = 6$) or 100 mg/kg/day ($n = 6$) or a vehicle control ($n = 6$) p.o. for 14 days. After euthanizing the mice on Day 19, tumor volume was measured and tumor samples and intraperitoneal lavage fluid were collected. The tumor samples were formalin fixed ($n = 3$) or stored in Isogen ($n = 3$) (Nippon Gene, Tokyo, Japan). The intraperitoneally disseminated cells were collected from 2 ml of PBS that had been used to wash the peritoneal cavity.

In the survival study, mice were inoculated with 1×10^6 58As1 or 44As3 cells into the gastric wall after laparotomy. Three days after inoculation, the mice were given ZD6474 50 mg/kg/day of ZD6474 p.o. ($n = 7$) or i.p. ($n = 7$) or the vehicle control p.o. ($n = 7$) for 14 days. The "visible ascites," which was evident a few days before death in this model, was used as a surrogate for survival time to consider for animal welfare. Mice were euthanized when ascites became visible, implantation of the gastric cancer cells was confirmed in all of the euthanized mice. No cancer cell was found in one mouse (ZD6474 100 mg/kg/day, 44As3 implanted), and it was excluded from the analysis. The experimental animal protocols were approved by the Committee for Ethics of Animal Experimentation, and the experiments were conducted in accordance with the Guidelines for Animal Experiments of the National Cancer Center.

Oligonucleotide microarray study

A DNA microarray procedure was used to prepare the *in vitro* transcription products, and oligonucleotide array hybridization and scanning were carried out according to the Affymetrix protocols (Santa Clara, CA). In brief, total RNA extracted from the tumor samples was analyzed with the Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) and cRNA was synthesized with the GeneChip® 3′-Amplification Reagents One-Cycle cDNA Synthesis Kit (Affymetrix). The cRNA were then labeled and purified for use as probes. Hybridization was carried out to the Affymetrix GeneChip HG-U133 Plus2.0 array for 16 hr at 45°C. After washing the glass slides, spot intensity was measured by scanning with a GeneChip® Scanner3000 (Affymetrix) and converted to numerical data with GeneChip Operating Software, Ver.1 (Affymetrix).

Six GeneChips were used to primary implanted 58as1 tumor samples from the vehicle control group ($n = 2$), and the ZD6474-treated group ($n = 2$, 50 mg/kg group; $n = 2$, the 100 mg/kg group).

Statistical analysis

All statistical calculations, except the analysis of the microarray data, were carried out using StatView version 5 software (SAS Institute Inc., Cary, NC). A p-value of <0.05 was considered significant. The microarray data were analyzed with GeneSpring software (Silicon Genetics, Redwood City, CA). The expression data were normalized across the sample set by the 50th percentile of each chip's intensity range. Relative expression data for each probe set were generated by median normalization. The fold change (mean value of the ZD6474-treatment group/mean value of the vehicle control group) was calculated, and genes with >2-fold change or <0.5-fold change were selected.

Results

Cell sensitivity to ZD6474 in vitro *and expression of VEGFR and EGFR*

Cell sensitivity to ZD6474 and the expression levels of EGFR, VEGFR and VEGF were examined in the 58As1 cells. The growth-inhibitory effect of ZD6474 and gefitinib was assessed by an MTT assay. The IC_{50} values of ZD6474 and gefitinib for 58As1 cells were 5.8 ± 1.8 and 11.0 ± 3.0 μM, respectively, suggesting that 58As1 cells are not sensitive to ZD6474 or gefitinib *in vitro*, compared to the "hypersensitive" PC-9 cells (IC_{50} values = 0.09 and 0.03 μM, respectively).[15] The 58As1 cells expressed a relatively high level of EGFR compared to the cells expressing high (PC-9) and low (HEK293) levels of EGFR, but the phosphorylation status was low (Fig. 1*a*). The expression levels of VEGFR and VEGF-A,C were measured by RT-PCR. A low

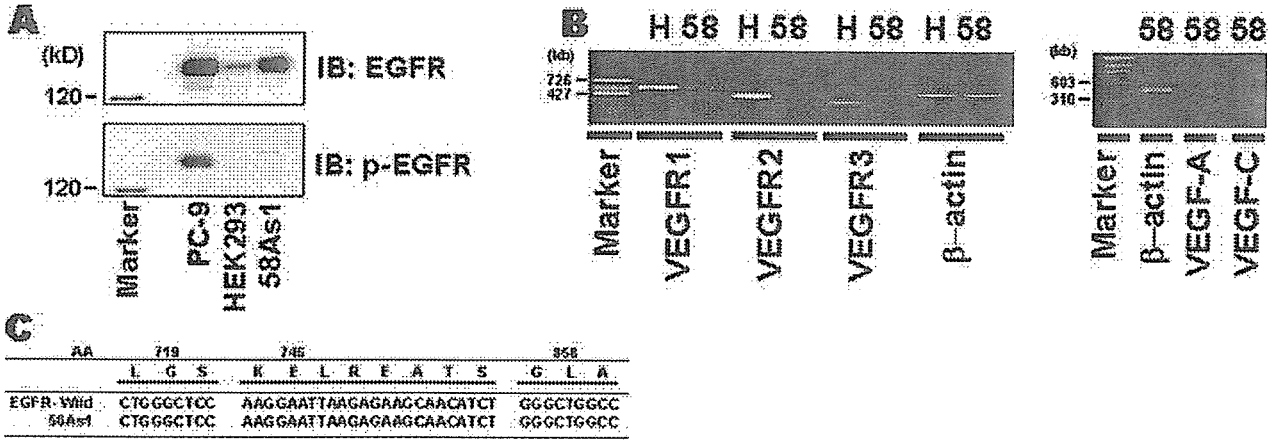

FIGURE 1 – Cellular characteristics of 58As1 cells. (*a*) EGFR expression and phosphorylation levels determined by Western blotting. A moderately high level of EGFR expression was observed in 58As1 cells, compared to cells expressing high (PC-9) and low (HEK293) levels of EGFR. The phosphorylation of EGFR status in 58As1 cells was low under normal culture conditions. IB, immunoblotting. Molecular marker: 120 kD. (*b*) Expression levels of VEGFR and VEGF-A and VEGF-C were measured by RT-PCR. A low level of VEGFR1 expression was detected in 58As1 cells, but no expression of VEGFR-2 or 3 was detected. 58As1 cells expressed VEGF-A but not VEGF-C. H, human umbilical vein endothelial cells. 58: 58As1. (*c*) EGFR sequence in 58As1 cells. No mutations were detected near the ATP-binding domains in 58As1 cells. AA, amino acid.

level of VEGFR1 expression was found in the 58As1 cells, but no VEGFR2 or VEGFR3 expression was detected. The 58As1 cells expressed VEGF-A, but not VEGF-C (Fig. 1*b*). Our results suggest that the lymphatic-metastasis-related VEGF-C and VEGFR3 are not involved in the inhibitory effect of ZD6474 on tumor dissemination observed in our present study *in vivo*.

Because EGFR mutations may be a determinant of tumor cell sensitivity to ZD6474,[15] exons 18–21 of EGFR mRNA from 58As1 cells were sequenced. No mutations near the ATP-binding domains[18,19] were detected, the 58As1 cells were concluded to express the wild-type EGFR.

Growth-inhibitory effect of ZD6474 in the orthotopic model in vivo

To examine the antitumor effect of ZD6474 on gastric cancer, we assessed the growth-inhibitory effect of ZD6474 by measuring implanted tumor volume after 14 days of p.o. treatment *in vivo*. A significant growth-inhibitory effect was observed in the ZD6474 (100 mg/kg/day) group in comparison with the vehicle control group ($p = 0.027$) in athymic mice implanted with 58As1 cells (Fig. 2*a*). Average tumor volume in the vehicle control group, 50 mg/kg/day ZD6474 group and 100 mg/kg/day ZD6474 groups was 106.3 ± 81.8 mm^3, 79.9 ± 70.0 mm^3, and 42.3 ± 24.8 mm^3, respectively.

Histological examination of H&E stained specimens showed a marked reduction in the number of cancer cells in the sub-mucosal lesions and the presence of necrotic tissue in the ZD6474 groups (Fig. 2*b*), suggesting that ZD6474 inhibits the growth of primary gastric tumor *in vivo*.

Inhibitory-effect of ZD6474 on peritoneal dissemination

To monitor the inhibitory effect of ZD6474 on peritoneally disseminated human cancer cells, the mRNA expression ratio of human β-actin/murine β-actin was measured with appropriate specific primers in cells collected from intraperitoneal lavage fluid. A significantly lower level of human-derived β-actin was observed in the 100 mg/kg/day ZD6474 group than in the vehicle control group ($p = 0.049$) (Fig. 2*c*,*d*), indicating that ZD6474 inhibits the intraperitoneal dissemination of gastric cancer in a dose-dependent manner.

Effect of ZD6474 on survival

In the survival experiment, we examined the effect of ZD6474 (p.o. or i.p.) on the survival of mice implanted with 58As1 or 44As3 cells. Both p.o. and i.p. administration of ZD6474 50 mg/kg/day significantly improved the survival of 44As3-implanted mice ($p = 0.0009$, $p = 0004$ *vs.* control, Fig. 3*b*), but did not significantly improve the survival of 58As1-implanted mice ($p = 0.09$, $p = 0.4$ *vs.* control, Fig. 3*a*). The median survival time of the 58As1-implanted mice was 33 days in the control group, 40 days in the i.p. group, and 46 days in the *p.o.* group, whereas in the 44As3-implanted mice, it was 34 days, 43 days and 53 days, respectively. Oral administration of ZD6474 was more effective than i.p. injection ($p = 0.049$) in the 44As3-implanted mice (Fig. 3*b*). These results suggest that ZD6474 is an active against gastric cancer.

Regulation of the gene expression by ZD6474 in vivo

To identify putative tumor biomarkers that reflect the efficacy of ZD6474 in vivo, we analyzed the gene expression profiles of implanted-tumor samples with oligonucleotide microarray. Expression of 26 genes was upregulated by 2-fold or more in the ZD6474 treatment group compared to the control group, whereas 2 genes were downregulated (Fig. 4*a*). Interestingly, of 26 upregulated genes, 9 of these genes were reported previously to be hypoxia-inducible: *IGFBP3* (*insulin-like growth factor binding protein 3*), *ADM* (*adrenomedullin*), *ANGPTL4* (*angiopoietin-like 4*), *PLOD2* (*procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2*), *DSIPI* (*delta sleep inducing peptide, immunoreactor*), *ENO2* (*enolase 2*), *NDRG1* (*N-myc downstream regulated gene 1*) *HIG2* (*hypoxia-inducible protein 2*) and *BNIP3L* (*BCL2/adenovirus E1B 19 kDa interacting protein 3-like*). To confirm upregulation of the genes, we measured the expression levels of representative genes, *IGFBP3* and *ADM*, by RT-PCR in murine tumor samples (Fig. 4*b*).

Discussion

A correlation between somatic EGFR mutations in non-small cell lung cancer cells and sensitivity to EGFR-specific tyrosine kinase inhibitors, including gefitinib and erlotinib, has been demonstrated recently,[18–20] and a similar observation was made in regard to ZD6474 *in vitro*.[15] We demonstrated previously that cells transfected with mutated EGFR were ~60-fold more sensitive to ZD6474 *in vitro*. EGFR tyrosine kinase inhibitors may pro-

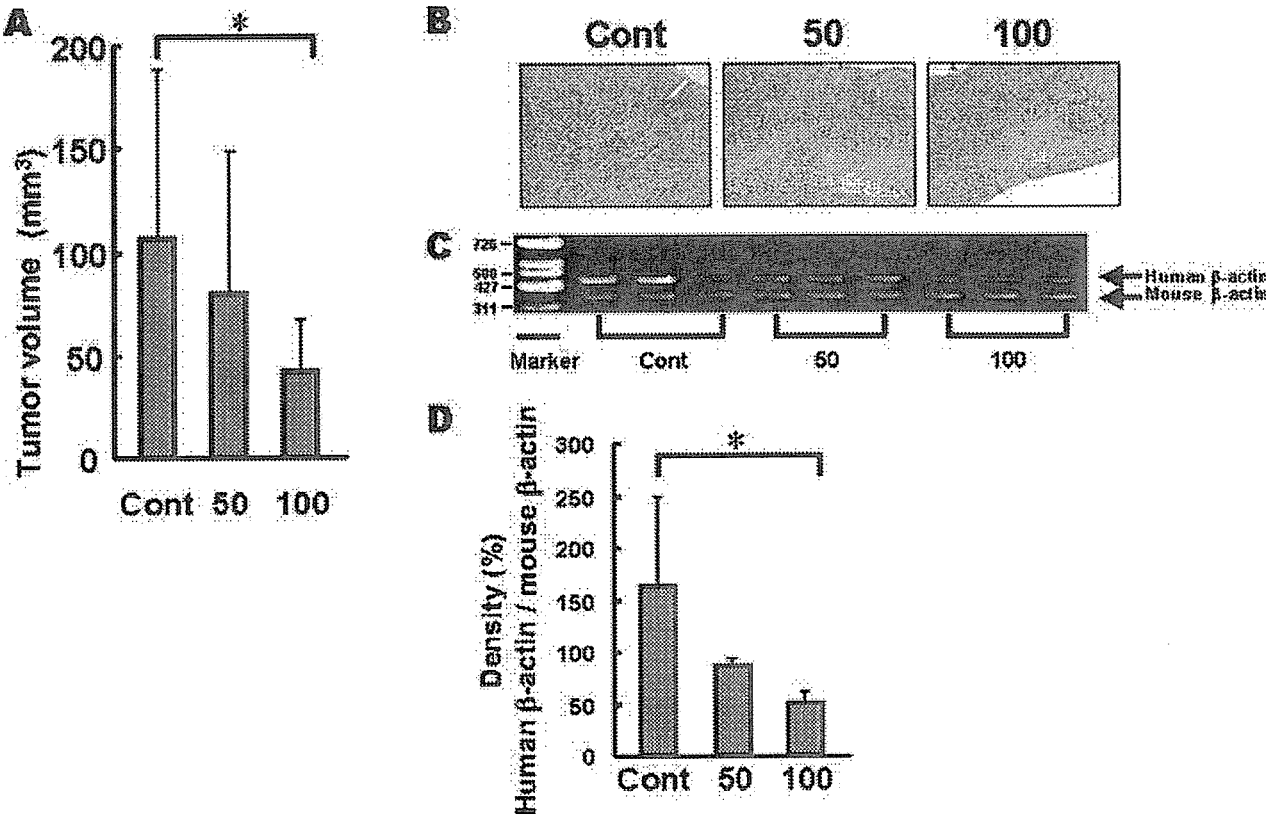

FIGURE 2 – Antitumor effect of ZD6474 in an orthotopic dissemination model *in vivo*. (*a*) *In vivo* growth-inhibitory effect of ZD6474. The implanted tumor volume was calculated after 14 days of treatment (p.o.). *Athymic mice (n = 7) per group were implanted with 58As1 cells, and a significant growth inhibitory effect was observed in the 100 mg/kg/day group, compared to the vehicle control group (p = 0.027). (*b*) Representative HE staining of tumor tissue in mice treated with ZD6474. The number of cancer cells in the sub-mucosal lesions was clearly lower and necrotic tissue was visible in the ZD6474 group, compared to the control group. (*c*) Disseminated cancer cells were collected from intraperitoneal lavage fluid to measure the ratio of tumor-derived human β-actin to murine β-actin using RT-PCR and specific primers (3 mice/group). Total RNA was equalized to 5 μg for each sample. (*d*) Densitometric analysis. Ratio of β-actin levels. *Significantly lower level of human-derived β-actin was detected in the 100 mg/kg/day ZD6474 group than in the control group (p = 0.049). The data shown are means ± SD. Cont, vehicle control; 50, ZD6474 50 mg/kg/day group; 100, ZD6474 100 mg/kg/day group. Significance was analyzed by Student's t-test.

vide particularly effective therapy for the subset of lung cancer patients whose tumor cell growth is highly dependent on EGFR signaling, including patients with tumor cells harboring activated, mutated EGFR. The proportion of patients with tumors highly dependent on EGFR signaling may be relatively small among various cancer patient populations. Therefore, additional treatment options for patients with cancers less dependent on EGFR signaling are also needed. It was concluded that 58As1 cells expressing wild-type EGFR are not highly dependent on EGFR signaling *in vitro* because the IC_{50} for growth inhibition by ZD6474 (5.8 μM) fell within the range of sensitivity reported by others for tumor cells *in vitro* (2.7–13.5 μM)[10] and because the IC_{50} for growth inhibition by gefitinib, a highly selective EGFR tyrosine kinase inhibitor, was >10μM. As a result, the concentration of ZD6474 required to inhibit 58As1 cell growth *in vitro* was 97-fold greater than required to inhibit VEGF-dependent HUVEC proliferation.[10] Nonetheless, ZD6474 significantly inhibited 58As1 tumor growth *in vivo* (Fig. 2*a*), suggesting that ZD6474 is active against gastric cancers expressing wild-type EGFR *in vivo* and that ZD6474 inhibition of tumor angiogenesis is likely to contribute significantly to this antitumor effect.

Our present study is unique because our aggressive and spontaneous intraperitoneal-dissemination model is considered a very good model of tumor progression in gastric cancer patients clinically, especially of the undifferentiated-type. Indeed, Paclitaxel and CPT-11 have been demonstrated to exhibit a growth-inhibitory effect and survival benefit in this model,[17] but gefitinib did not in preliminary result (data not shown). The positive results with ZD6474 in our present study suggest that this agent may be clinically useful in gastric cancer. We had hypothesized that direct intraperitoneal injection of ZD6474 is more effective than oral administration to inhibit intraperitoneal dissemination and result in better survival, however, the result showed that oral administration led to better survival in 44As3-inplanted mice (Fig. 3*b*).

ZD6474 inhibited the intraperitoneal dissemination of gastric cancer cells in our dissemination model. Although the mechanisms underlying this effect remain unclear, a few possibilities can be speculated: based on clinical evidence, the depth of tumor invasion and clinical staging is thought to be closely related to peritoneal dissemination.[21] Thus, one possible mechanism of the reduction of intraperitoneal dissemination may have resulted from a reduction in the serosal penetration of the cancer cells by "antitumor effect of ZD6474" on the implanted tumors. Although it is unclear whether ZD6474 has an inhibitory effect against distal metastasis to the liver and lymph nodes, for examples, it is not surprising that ZD6474 inhibits "intraperitoneal dissemination." Evaluation of its effect on distal metastasis will be the subject of a future investigation in our laboratory. Small tumor lesions (up to 2 mm) may obtain the oxygen and nutrients

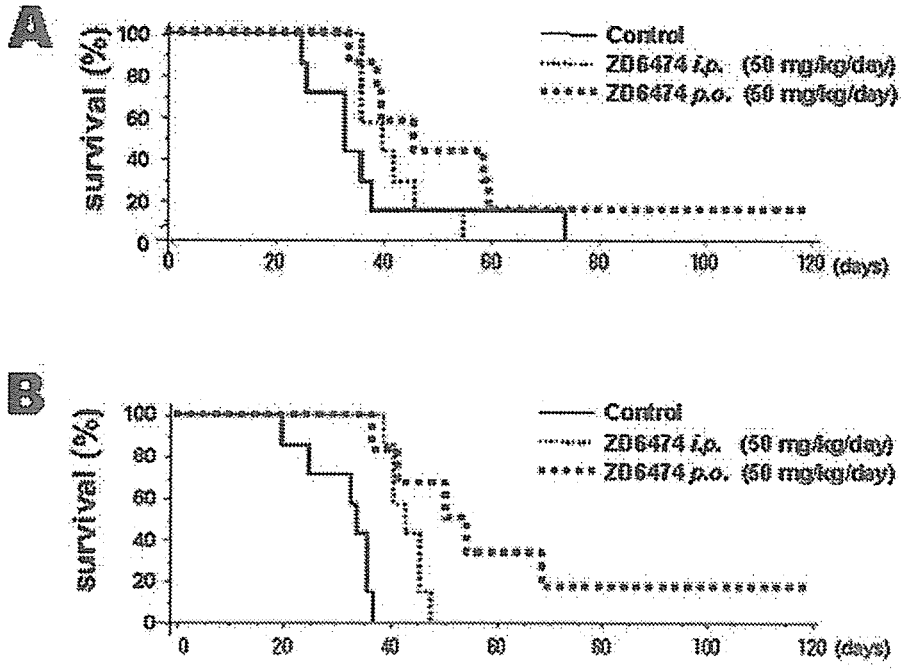

FIGURE 3 – Survival curve of 58As1 cells- (*a*) and 44As3 cells- (*b*) implanted mice treated with ZD6474. Both p.o. and i.p. administration of ZD6474 50 mg/kg/day significantly improved the survival of 44As3-implanted mice ($p = 0.0009$, $p = 0004$ *vs.* control), but did not significantly improve the survival of mice implanted with 58As1 cells ($p = 0.09$, $p = 0.4$ *vs.* control). The p values were calculated by the log-rank test.

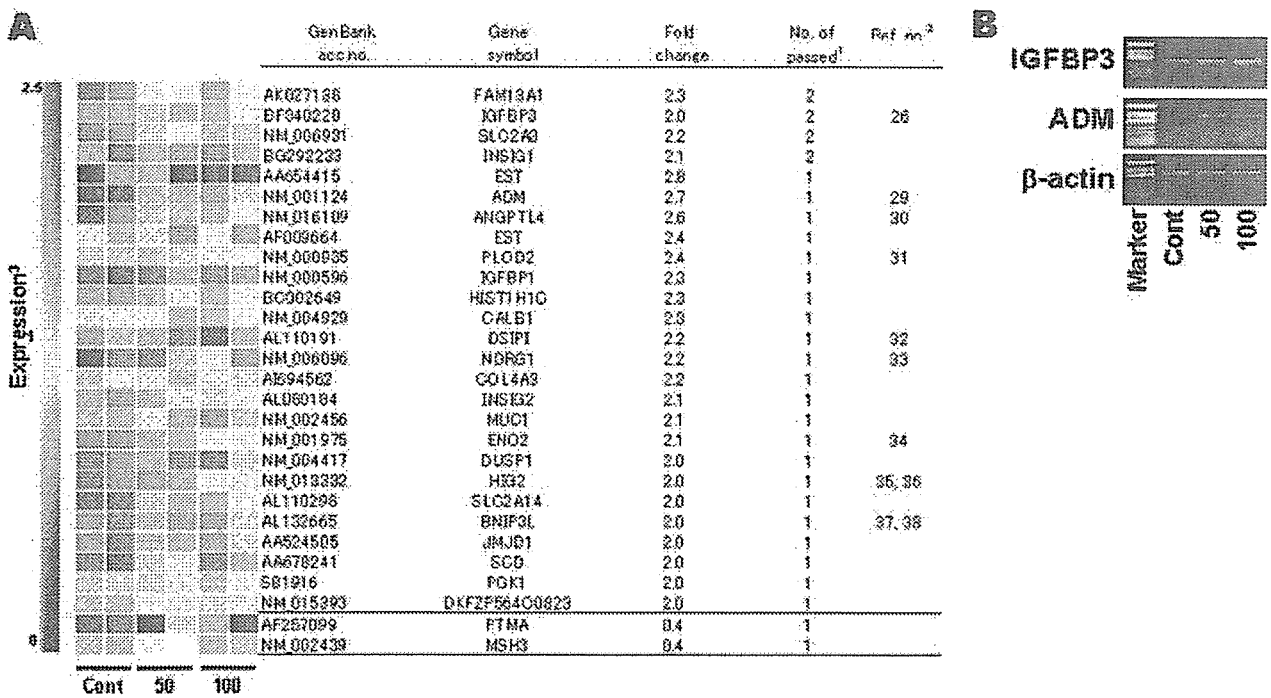

FIGURE 4 – Candidate genes for biomarkers regulated by ZD6474 treatment. Each colored block represents the expression level of a given gene in an individual sample. (*a*) Upregulated genes with a >2-fold change or <0.5-fold change are shown (mean value in the ZD6474 group/vehicle control group). Cont, vehicle control group, $n = 2$; 50, ZD6474 50 mg/kg/day group, $n = 2$; 100, ZD6474 100 mg/kg/day group, $n = 2$. [1]Number of different probes that passed fold-change criteria above. [2]Reference number for genes whose expression has been reported to be related to hypoxia. [3]Red represents increased expression and blue represents decreased expression relative to the normalized expression of the gene across all samples. (*b*) mRNA expression levels of 2 representative genes, *IGFBP-3* and *ADM*, detected by RT-PCR in tumors treated with ZD6474. *IGFBP-3* and *ADM* mRNA expression was induced in response to ZD6474.

they need by passive diffusion, but angiogenesis is required for the growth of tumors larger than 2 mm.[22] A second possible mechanism is that ZD6474 may inhibit the growth or migration of tumor vascular endothelial cells in "small tumor lesions" by inhibiting VEGFR2-dependent intracellular signaling. This effect would be expected to limit metastatic tumor growth due to lack of oxygen and nutrients, and reduce the dissemination of cancer cells.

To identify putative biomarkers of the pharmacodynamic effects of ZD6474 *in vivo*, we identified 28 candidate genes from implanted 58As1 tumor samples by oligonucleotide microarray analysis (Fig. 4*a*). IGFBP-3 has multiple functions, including in the induction of apoptosis,[23] the inhibition of cancer cell proliferation,[24] and carcinogenesis[25] and IGFBP-3 expression is transcriptionally upregulated under hypoxic conditions.[26] A recent study has also shown that EGFR regulates IGFBP-3 expression and secretion.[27] The inhibitory effect of ZD6474 on EGFR kinase may be associated with the upregulation of IGFBP-3. ADM, which was first identified in a human pheochromocytoma, is known to regulate circulation by acting as a hormone.[28] Adrenomedullin is also induced by hypoxia and may have a role in protecting against hypoxic cellular damage in human retinal pigment epithelial cells.[29] Expressions of nine of the upregulated genes, *IGFBP-3*, *ADM*, *ANGPTL4*, *PLOD2*, *DSIPI*, *NDRG1*, *ENO2*, *HIG2* and *BNIP3L*, has been reported previously to be induced by hypoxia.[26,29–38] We hypothesize that ZD6474 inhibits neovascularization in tumors, thereby limiting the oxygen and nutrient supply and resulting in tumor hypoxia and upregulation of hypoxia-inducible genes. If this hypothesis is correct, hypoxia-regulated genes and gene products might be useful biomarkers for the pharmacodynamic effects of ZD6474 and other anti-angiogenic agents in preclinical and clinical settings. We are now investigating whether these genes and gene products can be used as biomarkers for the efficacy of ZD6474 in a correlative study.

Future directions of our study include: (*i*) to compare the antitumor effect of other "anti-vascular agents" with ZD6474 in this model; (*ii*) to evaluate combination therapy with ZD6474 plus anticancer agents; (*iii*) to evaluate the antitumor effect of ZD6474 against micro-metastasis *in vivo*; and (*iv*) to confirm the usefulness of the 9 candidate genes as biomarkers in clinically.

In conclusion, we demonstrated that ZD6474 inhibited tumor growth, suppressed intraperitoneal dissemination, and prolonged survival in a highly metastatic orthotopic gastric cancer model. We carried out a microarray analysis of tumor samples and we identified 9 hypoxia-inducible genes as candidate biomarkers for monitoring the effects of ZD6474 therapy. These findings provide a strong preclinical rationale for investigating ZD6474 for the treatment of gastric cancer in the clinic.

Acknowledgements

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特集／がん薬物療法の最前線

各種がんに対する薬物療法

乳がん薬物療法の現状と今後

青儀健二郎　　高嶋成光

はじめに

乳がん領域における薬物療法は，アンスラサイクリン系薬剤・タキサン系薬剤を中心とする抗がん剤療法と内分泌療法の2本柱を中心に展開されてきたが，近年これに加えるべき新規治療として，分子標的薬剤であるトラスツズマブ（ハーセプチン®）がある。その進行再発乳がんにおける有効性は証明されているが[1]，最近では，術後補助療法におけるトラスツズマブの有効性を示す臨床試験の結果が次々と報告されている。本稿では，これらのエビデンスを中心に，乳がん薬物療法の現状と今後の展開について述べてみたい。

Ⅰ．分子標的薬剤

分子標的薬剤とは，がん細胞の分子生物学的手法により明らかにされたがん細胞の増殖，浸潤，転移に関わる分子を標的として，その機能の失活や復元を行うことにより，がん治療を行うものである[2]。近年の分子生物学の進歩に伴い，多くの分子標的薬剤が開発されており，それらの一部は臨床試験においても有用性が示されている。分子標的薬剤の条件として，①正常細胞にはないがん細胞の分子生物学的特徴を標的とし，かつ修飾すること，②抗腫瘍効果が分子標的の修飾により生じていることが証明可能であること，がある。また，がんに対する選択性が高く，副作用も軽いことから，新規の治療薬として注目されている。

分子標的薬剤を標的によって分類すると，シグナル伝達系阻害剤，増殖因子受容体阻害剤，転写因子阻害剤，細胞周期関連蛋白阻害剤，浸潤・転移関連分子阻害剤，血管新生関連分子阻害剤，ホルモン受容体阻害剤などがあるが，今回は増殖因子受容体阻害剤のトラスツズマブ，血管新生関連分子阻害剤のベバシツマブを用いた臨床試験の結果を挙げる。

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臨床研究部

Ⅱ．トラスツズマブの臨床試験

増殖因子受容体ファミリーのひとつであるHER2受容体のマウス由来モノクローナル抗体であるトラスツズマブは，本邦においては2001年6月から，進行再発乳がんを適応として臨床の場に登場した。HER2の過剰発現が認められた転移性乳がん患者に対して，トラスツズマブ＋化学療法を化学療法単独と比較する海外の無作為化多施設第Ⅲ相臨床試験では，time to disease progression（TTP），生存期間の延長，奏効率の改善等が報告された[1]。加えてImmunohistochemistry（IHC）法において3+，またはFISH法において陽性であるHER2受容体陽性乳がん患者に対するトラスツズマブの術後補助療法においての有用性を示す大規模臨床試験の中間解析の結果についてNational Surgical Adjuvant Breast and Bowel Project（NSABP）B-31とIntergroup Trial N 9831の共同解析[3][4]，Herceptin Adjuvant(HERA) Trialの中間解析結果が2005年American Society of Clinical Oncology（ASCO）総会で[5][6]，Breast Cancer International Research Group（BCIRG）006の中間解析結果が2005年サンアントニオ乳がんシンポジウムで報告された[7]。

NSABP B-31はリンパ節転移陽性（stage ⅡB-ⅢA）のHER2陽性乳がん患者で，AC（anthracycline＋cyclophosphamide，60/600mg/m^2，4回）followed byパクリタキセル（175mg/m^2 3週毎投与，4回）群とAC followed byパクリタキセル＋トラスツズマブ（毎週投与，初回4mg/kg，2回目は2mg/kg，計51回）群の2群間で比較を行っている。またIntergroup Trial N 9831は同様の対象患者で，治療群はAC followed byパクリタキセル群，AC followed byパクリタキセル（80

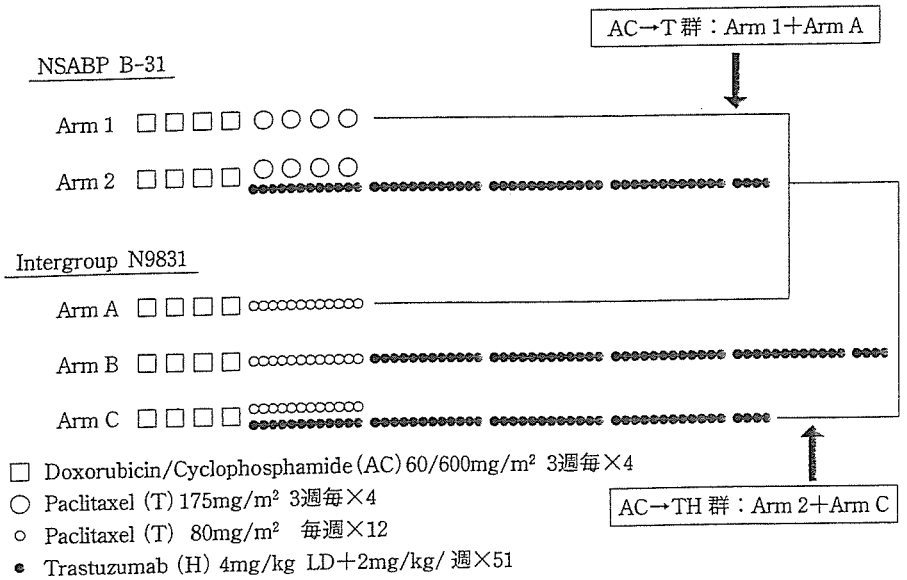

図 1　B-31/N9831 試験の共同解析

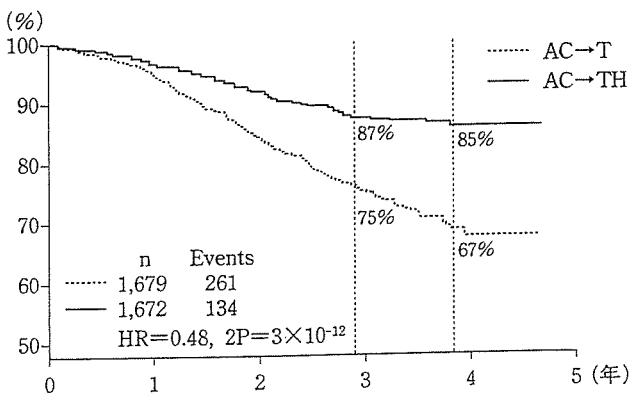

図 2　B-31/N9831 試験の DFS

mg/m² 毎週投与，12回）＋トラスツズマブ追加投与群，AC followed by パクリタキセル＋トラスツズマブ同時併用及び追加投与群の3群になっている。今回の発表では，2つの試験で同じ治療を行っている群同士を AC followed by パクリタキセル群（1,679人）対 AC followed by パクリタキセル＋トラスツズマブ群（1,672人）の2群に統合し，解析を行っている。プライマリーエンドポイントは disease-free survival (DFS)，セカンダリーは overall survival (OS)，有害事象である。その結果，治療後3年の DFS で HR＝0.48，2P＝3×10⁻¹²，OS で HR＝0.67，2P＝0.015 と有意にトラスツズマブを加えた群が良好であった。また Romond らは NSABP B-31 の解析結果から，トラスツズマブは33%の死亡リスク減に役立っているとした[4]。有害事象においては，うっ血性心不全の発現率が，B-31 においてトラスツズマブを加えた群（846人）において4.0%，加えない群（811人）において0.6%であった。

HERA Trial は，HER2 陽性乳がん患者に対し通常の補助療法終了後，無治療群，トラスツズマブ1年治療群，トラスツズマブ2年治療群の3群比較を行った，5,090人登録の大規模試験である。トラスツズマブは初回8 mg/kg，2回目は6 mg/kg で3週毎投与を行っている。今回の解析は，中央観察期間1年で無治療群（1,693人）とトラスツズマブ1年治療群（1,694人）の2群間について行われている。プライマリーエンドポイントは DFS，セカンダリーは，recurrence-free survival (RFS)，distant disease-free survival (DDFS)，OS，有害事象である。2年目の DFS において，トラスツズマブ1年治療群は無治療群に比べ，ハザード比0.54（0.43～0.67）(p＜0.0001）と良好であった。RFS はハザード比0.50（0.40～0.63）(p＜0.0001)，DDFS はハザード比0.51（0.40～0.66）(p＜0.0001）であったが，OS においては有意差は認めなかった（ハザード比0.76（0.47～1.23）(p＜0.26))。有害事象においてトラスツズマブ1年治療群には心臓死はないものの，左室駆出率の減少が10points 以上でかつ左室駆出率50%未満であった症例が7.1%と無治療群が2.2%であったのに比べ高かった。

BCIRG006 は，3,222名の HER2 陽性乳がんで

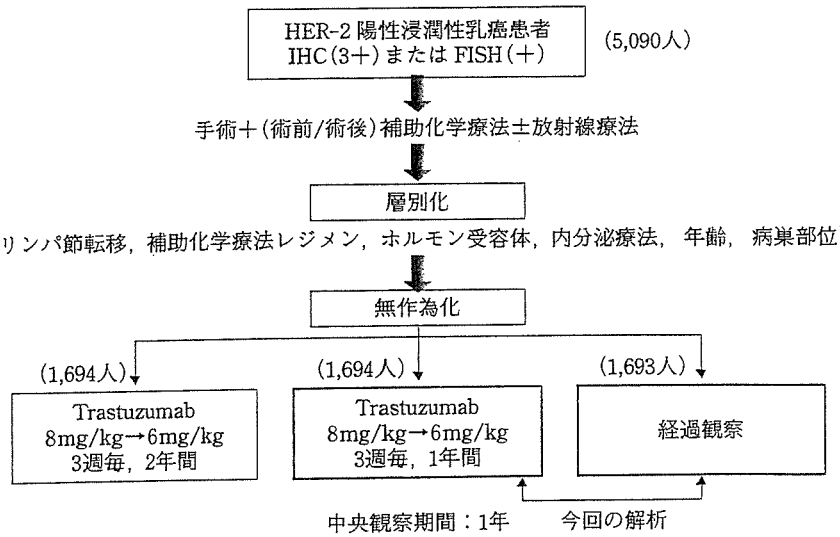

図　3　HERA　試　験

リンパ節転移陽性，もしくは陰性であるが高リスクの術後患者に対して，AC(60/600mg/m^2, 4回) followed by ドセタキセル (100mg/m^2, 4回) 治療 (ACT) 群 (1,073人)，AC followed by ドセタキセル＋トラスツズマブ1年間併用群(ACTH) (1,074人)，ドセタキセル (75mg/m^2)＋カルボプラチン (AUC6)(6回)＋トラスツズマブ1年間併用 (TCH) 群 (1,075人) の3群比較を行っている。プライマリーエンドポイントはDFS，セカンダリーはOS，有害事象，病理学的，分子学的マーカーの変化である。中間解析では，DFSにおいてACTH群はACT群に比べハザード比で0.49 (0.37～0.65) (p<0.0001)，TCH群はACT群に比べハザード比で0.61 (0.47～0.79) (p=0.0002) と，2つのトラスツズマブ併用群において有意に良好な結果が示された。2つのトラスツズマブ併用群の間には差は認められなかった。OSはデータ不足のため解析ができず，有害事象においてはNCI-CTC (National Cancer Institute-Common Toxicity Criteria) ver3.0のGrade 3以上の心血管系の事象 (左室機能不全，虚血性心疾患，不整脈) の発生頻度は，ACT群0.95%，ACTH群2.34%，TCH群1.33%で，ACT群とACTH群の間では有意差が認められた (p=0.016)。また左室駆出率の10%以上の低下を認めた患者は，ACT群9%，ACTH群17.3%，TCH群8%で，ACTH群はACT群，TCH群それぞれに比べ，有意に高かった (p=0.002，p<0.0001)。

これらの試験では，各種のevent-free survivalにおいて有効性が示されているが，トラスツズマブの併用による心臓関連の有害事象の長期的評価が必要である。またHERA studyにおいてはトラスツズマブ1年治療群と2年治療群の解析が2008年に行われる予定で，トラスツズマブ治療の至適投与期間を探るためにもその結果が待たれる。

Ⅲ．他の分子標的薬剤の臨床試験

他の分子標的薬剤として，血管新生関連分子ベバシツマブの試験結果が，2005年のASCO総会において示された (E2100試験)[8]。この試験はVEGF-Aを認識するヒト化モノクローナル抗体であるベバシツマブ (大腸がん領域においてはその有用性が証明されており，FDAにより承認も受けている) の乳がんにおける有用性を検証する目的で行われている。転移性乳がん患者に対し，パクリタキセル単独治療 (90mg/m^2, d1, d8, d15/28日間) 群と，パクリタキセル＋ベバシツマブ (10mg/kg, d1, d15) 併用治療群を比較するもので，両群併せて680人が適格で，パクリタキセル単独群339人，パクリタキセル＋ベバシツマブ併用群341人となっている。プライマリーエンドポイントはprogression-free survival (PFS)，セカンダリーは奏効割合，OS，有害事象である。PFSは，パクリタキセル単独群に比べ，パクリタキセル＋ベバシツマブ併用群は，ハザード比で0.51 (0.43～0.62)，Log rank testでp<0.0001と有意に良好であった。奏効割合は全患者で，パ

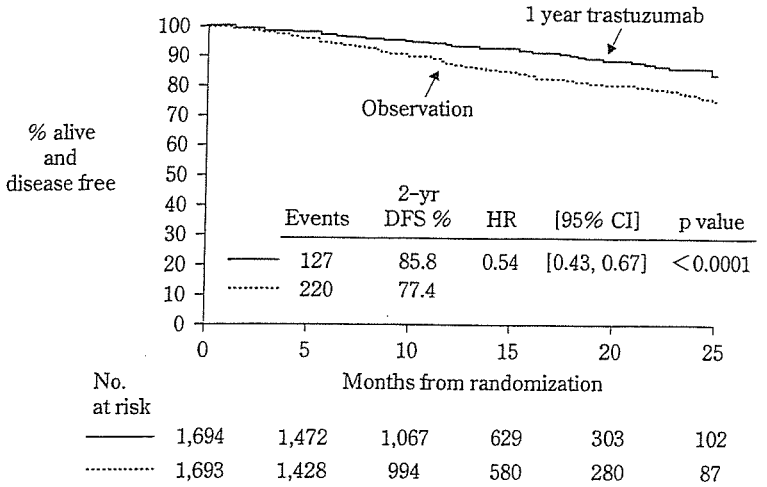

図　4　HERA　試　験　の　DFS

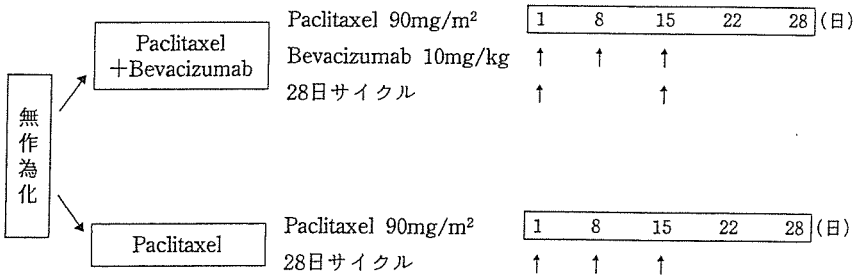

図　5　E2100　試　験

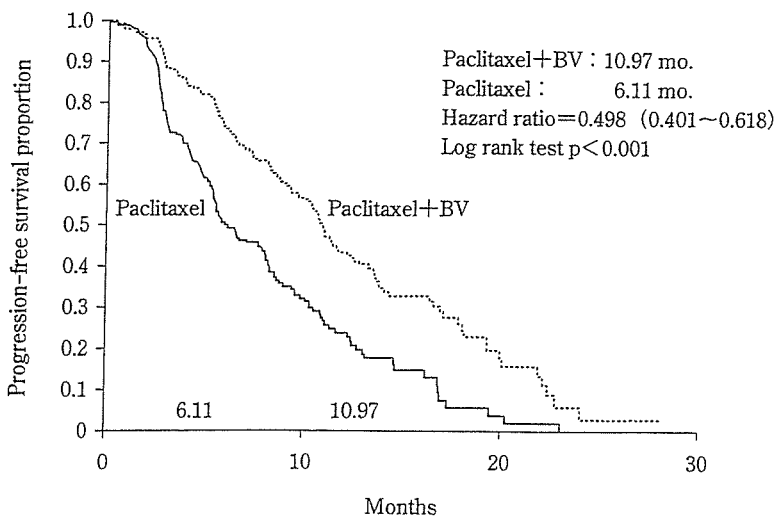

図　6　E2100　試　験　の　PFS

クリタキセル単独群13.8%であったのに対し，併用群で29.9%，計測可能病変を持つ患者では，単独群で16%，併用群で37.7%と，有意に良好であった（p<0.0001）。しかしOSでは2群間に有意差はなく（ハザード比0.84（0.64～1.05），Log rank testでp=0.12），有害事象においてNCI-

CTC ver3.0のGrade 3以上の全身倦怠感（p＝0.05），高血圧（p<0.0001）が併用群で認められているため，OS，有害事象に関してはさらに長期的なフォローアップが必要と考えられる。またベバシツマブが最も効果的な他薬剤との組み合わせ，スケジュール等，検討していくべき課題も数多くあると考えられる。

Ⅳ．分子標的薬剤療法の今後の展開

これらの分子標的薬剤は，現在臨床の場で用いられているトラスツズマブの有用性をみるまでもなく，今後乳がん治療の3本目の柱となり得るであろう。今後は，より有効性の高い薬剤の開発，分子標的薬剤と抗がん剤のより効率的な併用レジメンや分子標的薬剤同士の併用療法の開発，至適投与期間の確定，を行っていく必要があろう。また分子標的薬剤を用いる際には，治療の費用の問題はやはり看過できない。患者へのより有用な治療提供のためにもクリアすべき課題であろう。

おわりに

分子標的薬剤の最新のエビデンスについて概説した。解決すべき問題は山積しているが，その有効性はぜひ患者の治療において活かしていきたい。

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分子標的療法の実際

乳癌に対する分子標的療法

Advances in biological therapy for breast cancer

特集

青儀健二郎 *AOGI Kenjiro*　高嶋 成光* *TAKASHIMA Shigemitsu*

ここまできた分子標的療法

Key words 分子標的療法 トラスツズマブ ベバシツマブ

原発乳癌に対する治療としては，全身療法である薬物療法と，局所療法である手術および放射線療法の組み合わせが日常的に行われている．また再発乳癌において，薬物療法は治療の主体となっている．この薬物療法は，アンスラサイクリン系とタキサン系薬剤を中心とする抗癌剤療法と，タモキシフェンを中心とした内分泌療法の2本柱を中心に展開されてきた．しかし，近年この2つに加えるべき薬剤として，分子標的薬剤が登場した．代表的な薬剤としては，増殖因子受容体ファミリーのHER2受容体のマウス由来モノクローナル抗体であるトラスツズマブ(ハーセプチン®)である．トラスツズマブの進行再発乳癌における有効性は証明されているが[1]，最近では，術後補助療法における有効性を示す臨床試験の結果が次々と報告されている．乳癌に対する種々の分子標的薬剤の新しいエビデンスについて述べてみたい．

分子標的薬剤

分子標的薬剤は，分子生物学的手法により明らかにされた癌細胞の増殖，浸潤，転移に関わる分子を標的として，その機能の失活や復元を行うことにより，癌治療を行うものされている[2]．その特徴として，癌に対する選択性が高く，かつ副作用が軽いことがあげられ，近年注目されている．

分子標的薬剤をその分子生物学的標的によって分類すると，シグナル伝達系阻害剤，増殖因子受容体阻害剤，転写因子阻害剤，細胞周期関連蛋白阻害剤，浸潤・転移関連分子阻害剤，血管新生関連分子阻害剤，ホルモン受容体阻害剤などがある．本稿では増殖因子受容体阻害剤(トラスツズマブ)と血管新生関連分子阻害剤(ベバシツマブ)を用いた臨床試験の結果を示す．

独立行政法人国立病院機構四国がんセンター乳腺科・臨床研究部 *院長

トラスツズマブの術後補助療法における有用性

トラスツズマブは，本邦においては2001年6月から，HER2受容体陽性の進行再発乳癌を適応として承認され，臨床の場でも広く用いられている．Slamonら[1]は，HER2受容体の過剰発現のある転移性乳癌患者に対して，化学療法単独とトラスツ

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ズマブ＋化学療法とを比較する第Ⅲ相臨床試験において，time to disease progression(TTP)，生存期間の延長，奏効率の改善が認められたと報告している．

近年，Immunohistochemistry(IHC)法において3＋か，fluorescent *in situ* hybridization(FISH)法において陽性のHER2陽性乳癌患者に対するトラスツズマブの術後補助療法における有用性が示された臨床試験の中間解析結果，すなわちNational Surgical Adjuvant Breast and Bowel Project(NSABP) B-31とIntergroup Trial N 9831の共同解析(図1)[3]，Herceptin Adjuvant(HERA) Trial(図2)の中間解析結果が2005年ASCO総会において[4][5]，またBreast Cancer International Research Group(BCIRG)006(図3)の中間解析結果が2005年サンアントニオ乳癌シンポジウムにおいて相次いで報告された[6]．

NSABP B-31はリンパ節転移陽性(stageIIB-IIIA)のHER2陽性乳癌患者で，AC(anthracycline＋cyclophosphamide，60/600mg/m^2，4回) followed byパクリタキセル(175mg/m^2 3週毎投与，4回)群とAC followed byパクリタキセル＋トラスツズマブ(毎週投与，初回4mg/kgで2回目以降は2mg/kg，計51回投与)群の2群間で比較している．またIntergroup Trial N 9831は同様の対象患者で，治療群はAC followed byパクリタキセル群，AC followed byパクリタキセル(80mg/m^{2}毎週投与，12回)＋トラスツズマブ追加投与群，AC followed byパクリタキセル＋トラスツズマブ同時併用および追加投与群の3群になっている．今回2つの試験で同じ治療群をAC followed byパクリタキセル群(1,679人)対AC

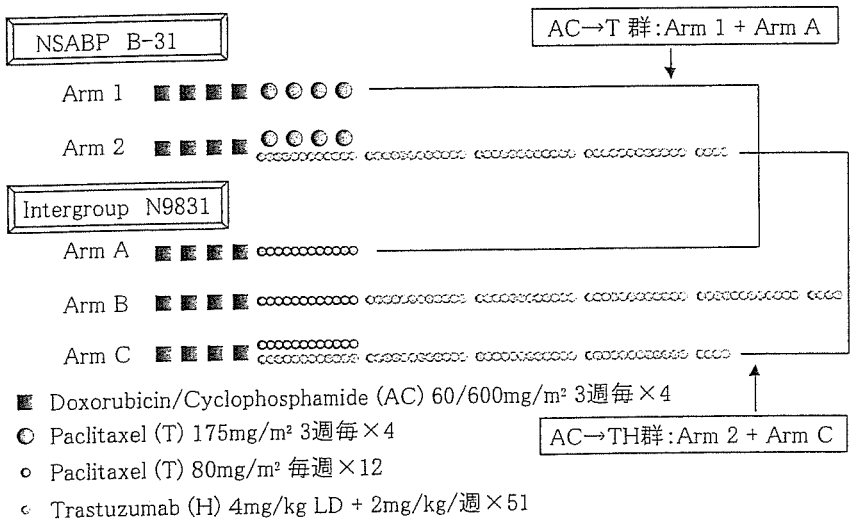

図1 B-31/N9831試験の共同解析

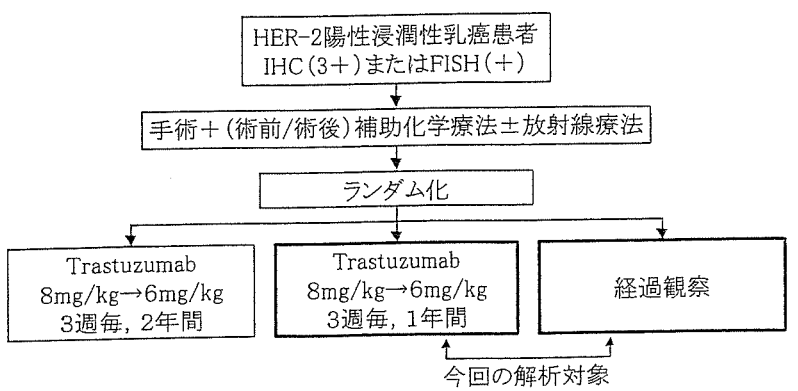

図2 HERA試験

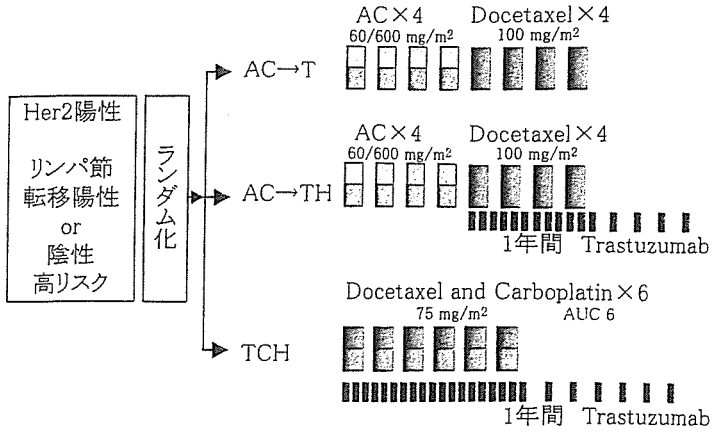

図3 BCIRG006試験

followed by パクリタキセル＋トラスツズマブ群(1,672人)の2群として統合解析した．プライマリーエンドポイントは disease-free survival (DFS)，セカンダリーは overall survival (OS)，有害事象である．その結果，治療後3年のDFSでハザード比0.48，$2P = 3 \times 10^{-12}$，OSでハザード比0.67，2P＝0.015と有意にトラスツズマブを加えた群が良好であった．有害事象においては，うっ血性心不全の発現率が，B-31においてトラスツズマブを加えた群(846人)において4.0%，加えない群(811人)において0.6%であった．

HERA Trialは，通常の補助療法終了後，無治療群，トラスツズマブ1年治療群，トラスツズマブ2年治療群の3群比較で，5,090人が登録されたglobalな大規模試験である．トラスツズマブは初回8 mg/kg，2回目以降は6 mg/kgで3週毎投与を行っている．今回，中央観察期間1年で無治療群(1,693人)とトラスツズマブ1年治療群(1,694人)の2群間について解析されている．プライマリーエンドポイントはDFS，セカンダリーは，recurrence-free survival (RFS)，distant disease-free survival (DDFS)，OS，有害事象である．2年目のDFSにおいて，トラスツズマブ1年治療群は無治療群に比べ，ハザード比0.54 (0.43-0.67) (p＜0.0001) と良好であった．RFSはハザード比0.50 (0.40-0.63) (p＜0.0001)，DDFSはハザード比0.51 (0.40-0.66) (p＜0.0001) であったが，OSにおいては有意差を認めなかった (ハザード比0.76 (0.47-1.23) (p＜0.26))．有害事象においてトラスツズマブ1年治療群には心臓死はないものの，EF値減少が10points以上でかつ左室駆出率50%未満であった症例が7.1%と無治療群が2.2%であったのに比べ高かった．

BCIRG006は，HER2陽性乳癌でリンパ節転移陽性，もしくは陰性であるが高リスクの術後患者に対して，AC (60/600mg/m², 4回) followed by ドセタキセル (100mg/m², 4回) 治療 (ACT) 群(1,073人)，AC followed by ドセタキセル＋トラスツズマブ1年間併用群 (ACTH) (1,074人)，ドセタキセル (75mg/m²) ＋カルボプラチン (AUC6) (6回) ＋トラスツズマブ1年間併用 (TCH) 群(1,075人)の3群比較を行っている．プライマリーエンドポイントはDFS，セカンダリーはOS，有害事象，病理学的および分子学的マーカー変化である．中間解析では，DFSにおいてACTH群はACT群に比べハザード比で0.49 (0.37-0.65) (p＜0.0001)，TCH群はACT群に比べハザード比で0.61 (0.47-0.79) (p＝0.0002) と，2つのトラスツズマブ併用群において有意に良好な結果が示された．2つのトラスツズマブ併用群の間には差は認められなかった．OSはデータ不足のため解析ができず，有害事象においてはNational Cancer Institute-Common Toxicity Criteria (NCI-CTC) ver3.0のGrade3以上の心血管系の事象 (左室機能不全，虚血性心疾患，不整脈) の発生頻度は，ACT群0.95%，ACTH群2.34%，TCH群1.33%

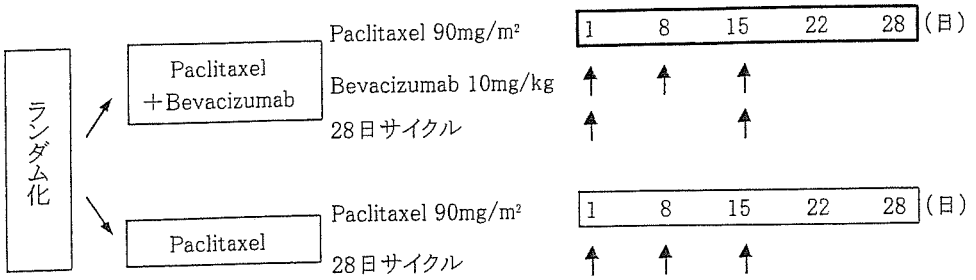

図4 E2100試験

で，ACT群とACTH群の間では有意差が認められた(p=0.016)．また左室駆出率の10%以上の低下を認めた患者は，ACT群9%，ACTH群17.3%，TCH群8%で，ACTH群はACT群，TCH群それぞれに比べ，有意に高かった(p=0.002, p<0.0001)．

これらの試験では，各種event-free survivalにおいてトラスツズマブの有効性が示されているものの，トラスツズマブ併用による心臓関連有害事象の長期的な評価は必須である．また今後HERA studyにおいて，トラスツズマブ1年治療群と2年治療群の比較解析が2008年に行われるが，トラスツズマブの至適投与期間を探るため，その解析結果は重要である．またHER1受容体とHER2受容体同時に阻害する経口dual inhibitorであるラパチニブも，現在国内外で臨床試験が展開されており，今後その結果が注目される．

ベバシツマブの臨床試験

腫瘍細胞の増殖・進展には新生血管は重要な役割を果たすことは容易に理解できる．この血管新生を促進する因子の1つに血管内皮増殖因子(vascular endotherial growth factor；VEGF)がある．このうちVEGF-Aを認識するヒト化モノクローナル抗体であるベバシツマブは転移性大腸癌において有効性が証明されている[7]．そのベバシツマブの乳癌における有効性を示した試験が，2005年のASCO総会において報告された(E2100試験，図4)[8]．転移性乳癌患者に対し，パクリタキセル単独治療(90mg/m², d1, d8, d15/28日間)群と，パクリタキセル+ベバシツマブ(10mg/kg, d1, d15)併用治療群を比較するもので，パクリタキセル単独群339人，パクリタキセル+ベバシツマブ併用群341人の2群の比較となっている．プライマリーエンドポイントはprogression-free survival(PFS)，セカンダリーは奏効割合，OS，有害事象である．PFSは，パクリタキセル単独群に比べ，パクリタキセル+ベバシツマブ併用群は，ハザード比で0.51 (0.43-0.62)，Log rank testでp<0.0001と有意に良好であった．奏効割合は全患者で，パクリタキセル単独群13.8%であったのに対し，併用群で29.9%，計測可能病変を持つ患者では，単独群で16%，併用群で37.7%と，有意に良好であった(p<0.0001)．しかしOSでは2群間に有意差はなく(ハザード比0.84 (0.64-1.05)，Log rank testでp=0.12)，有害事象においてNCI-CTC ver3.0のGrade3以上の全身倦怠感(p=0.05)，高血圧(p<0.0001)が併用群で認められているため，OS，有害事象に関してはさらに長期的な経過観察が必要である．

Millerら[9]は，アンスラサイクリンおよびタキサン既治療の転移性乳癌患者において，カペシタビン単独療法群(2,500mg/m², d1-14q3wks) (230人)とベバシツマブ(15mg/kg, d1)+カペシタビン併用療法群(232人)の2群比較を行った第3相比較試験の結果も報告している．プライマリーエンドポイントはPFSと有害事象，セカンダリーは奏効割合，奏効期間，OS，Quality of lifeである．ベバシツマブ併用により奏効割合は増加したものの(19.8% vs 9.1%, p=0.001)，PFS(4.86月 vs 4.17月，ハザード比0.98)，OS(15.1月 vs

14.5月)において差は認められなかった．ベバシツマブ併用群で治療を必要とする高血圧の頻度が増加していた(17.9% vs 0.5%)．

最も効果的な他薬剤との組み合わせ，スケジュール等，検討していくべき問題も数多く残されているが，再発乳癌患者に対するベバシツマブ療法は，適切な併用薬剤を選択することにより，有効な治療法として期待される．

分子標的薬剤療法の今後

分子標的薬剤は，今後乳癌治療の3本目の柱，それも大きな柱となり得る．より有効性の高い薬剤の開発，分子標的薬剤と抗癌剤のより効率的な併用レジメンや分子標的薬剤同士の併用療法の開発，効果予測因子の確定，至適投与期間の確定，長期的な有害事象，など検討すべき問題は山積している．さらに分子標的薬剤を用いる際には，費用も大きな問題であり，今後医療経済学的な検討が必要である．さらに，本邦においても分子標的薬剤を用いた臨床試験を行い，世界に対してエビデンスを発信していきたい．

結　　語

分子標的薬剤の最新のエビデンスについて概説した．今後出てくる臨床試験の結果に注目したい．

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