

## Report

# Report on the use of non-clinical studies in the regulatory evaluation of oncology drugs

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**Progress of Cancer Biology is Closely Linked to Oncology Drug Development**

The history of the development of oncology drugs, so-called chemotherapeutic agents, is closely associated with the progress of the biological understanding of cancer. Based on the concept that cancer cells are capable of unlimited proliferation, substances that inhibit DNA replication or cell division have been used as drugs for cancer treatment for a long period, since the 1950s. Although the concept has remained unchanged to the present day,<sup>(1)</sup> the discovery of cancer cell-specific

Non-clinical studies are necessary at each stage of the development of oncology drugs. Many experimental cancer models have been developed to investigate carcinogenesis, cancer progression, metastasis, and other aspects in cancer biology and these models turned out to be useful in the efficacy evaluation and the safety prediction of oncology drugs. While the diversity and the degree of engagement in genetic changes in the initiation of cancer cell growth and progression are widely accepted, it has become increasingly clear that the roles of host cells, tissue microenvironment, and the immune system also play important roles in cancer. Therefore, the methods used to develop oncology drugs should continuously be revised based on the advances in our understanding of cancer. In this review, we extensively summarize the effective use of those models, their advantages and disadvantages, ranges to be evaluated and limitations of the models currently used for the development and for the evaluation of oncology drugs.

metabolic pathways has led to the development of antimetabolites.<sup>(2)</sup> After the discovery of cancer cell-specific molecular and cellular mechanisms that are essential for the survival and growth of cancer cells, therapeutic drugs targeting these mechanisms, so-called molecular targeted drugs, started to be developed.<sup>(3)</sup> Research into viral oncogenesis, started in the 1960s, led to the discovery of oncogenes,<sup>(4)</sup> and research into the genetic backgrounds of cancers led to the discovery of tumor suppressor genes.<sup>(5)</sup> In the course of such studies, it also became apparent that cancer is caused by genetic abnormalities such as mutations, deletions, duplications, and translocations.<sup>(6–9)</sup> Molecular targeted cancer drugs appeared in the 1990s;<sup>(10)</sup> can-

cer was considered a disease characterized by abnormal differentiation, and the efficacy of differentiation-inducing agents was demonstrated.<sup>(11,12)</sup> Furthermore, it was shown that a solid tumor tissue consists of cancer and host cells such as vascular cells, fibroblasts, and cells in the immune system and that these host cells are essential for tumor growth. Drugs targeting the function of these host cells and their interactions with cancer cells were proven to be effective.<sup>(13)</sup> Based on these findings, it has been thought that regulatory mechanisms for the entire organism are involved in the action of oncology drugs that regulate the immune system.<sup>(14)</sup>

### Significance of Non-Clinical Studies in Efficacy Evaluation and Safety Prediction

Non-clinical studies are necessary at each stage of the development of oncology drugs. Particularly, the efficacy and the safety of a drug must be examined and evaluated before undertaking any clinical study of the drug. Types of non-clinical studies and how critical they are vary depending on the types and mechanisms of action of oncology drugs. Non-clinical studies required to develop drugs targeting cancer–host interactions differ markedly from those on substances having direct killing effects on cancer cells. Many experimental cancer models (animal models, *ex vivo* models, and *in vitro* models) have been developed to investigate carcinogenesis, cancer progression, metastasis, and other aspects in cancer biology. These models turned out to be useful in the efficacy evaluation and the safety prediction of oncology drugs. The present review summarizes the effective use of those models, their advantages and disadvantages, ranges to be evaluated, and limitations of the models used in non-clinical study.

### Evaluation of Oncology Drugs Using Experimental Animal Models

Two classes of experimental animal models for human cancers are currently used for the evaluation of oncology drugs: transplantation models and autochthonous cancer models. Transplantation models have been playing an important role in the non-clinical evaluation of oncology drugs. They are generally categorized into two types, namely xenograft models using human cancer cells and orthograft models using murine cancer cells. There has been some debate that the efficacy evaluation of oncology drugs in transplantation models might not be adequate for predicting the clinical efficacy or the types of cancer for which the drug could be effective. As autochthonous cancer models, chemical carcinogen-induced models were first established and the subsequent technological progress in gene manipulation allowed researchers to produce models harboring the genetic mutations of human cancer. Although a number of technical issues regarding the ability to maximize the utility of these models need to be addressed, such as their usability, reproducibility, and throughput compared with transplantation models, autochthonous cancer models clearly show some promise. In Table 1, we summarize the characteristics of those experimental cancer models used to evaluate the efficacy of oncology drugs in non-clinical studies.

**Transplantation cancer models.** In general, the s.c. (heterotopic) transplantation models with cancer cell lines have been used, and the efficacies of oncology drug response are evaluated based on tumor size. These models are particularly useful when a drug has a marked antiproliferative effect on cancer

cells. It is also easy to access tumor tissue samples from these models for subsequent pharmacodynamic evaluations. Despite such clear advantages, these models may not reflect the actual characteristics of the cancer microenvironment because the s.c. tissue is “heterotopic” for most cancer cells. In this context, orthotopic transplantation models may reproduce the cancer microenvironment more faithfully, although their utility caused by species differences should be considered. To analyze metastasis dissemination of cancer cells, experimental metastasis models have been considered as useful for evaluating drug efficacy in the process after the invasion of cancer cells from the primary tumor into the nearby blood vessel. Although these models have clear advantage in their usability and reproducibility, they cannot reproduce the entire step before the extravasation of cancer cells and may not accurately represent actual metastases by injecting a substantial number of cancer cells into the blood vessel. In this regard, spontaneous metastasis models have been considered to reflect the process of the metastasis of cancer cells more accurately than the heterotopic or orthotopic transplantations. Despite the clear advantages of these models, only a limited number of cancer cell lines are available and the results of experiments often vary. In addition to the above transplantation cancer models with cancer cell lines, patient-derived xenograft models have been considered as emerging animal models recapitulating the clinical condition of individual cancer patients, and therefore attracted much attention on precision treatment.<sup>(15–17)</sup>

**Autochthonous cancer models.** There are two major types of autochthonous cancer models, carcinogen-induced models and gene-engineered mouse (GEM) models. Of these, GEM models have been regarded as a better choice for testing drug efficacy, because the drug effects can be evaluated on autochthonous cancer cells induced by gene mutations resembling human cancer. As summarized in Table 2, there are several pros and cons to using autochthonous cancer models for drug efficacy tests in non-clinical studies. In particular, the timing of tumor occurrence and tissue specificity are often the major concerns of carcinogen-induced models and conventional knockout/transgenic mice. To overcome these issues, conditional gene knockout or gene expression technology provide us with the opportunity to use GEM models that more closely represent the pathology of human cancers. In addition to the above technical difficulties, the administrative challenges, such as maintenance of mouse strains to acquire a sufficient number of mice as well as the characters of each mouse model, including the latency and incidence of tumor and other relevant issues, need to be considered before undertaking efficacy studies testing oncology drugs in GEM models. Nevertheless, new technologies, such as *in vivo* imaging methods for small animals, have been introduced as powerful tools for quantitative evaluation of cancer occurrence and subsequent growth in GEM models. In Table 3, GEM models developing tumors induced by genetic mutations found in corresponding human cancers are summarized.

**Spontaneous cancer models using companion animals.** Even in companion animals, such as dogs and cats, the incidence of cancer has been increasing, likely due to their life extension together with genetic factors. In fact, cancer has become the leading cause of death among those companion animals. In particular, it has been known that the mortality from cancer is reported to be 47% (based on the report by the Veterinary Cancer Society, <http://www.vetcancersociety.org/members/>) in large breed dogs aged 10 years or more. Therefore, the establishment of early diagnosis methods and the development of

**Table 1. Characteristics of preclinical animal models for oncology drug development**

Model	Outline	Advantage	Disadvantage
Mouse cancer model	Transplantation model	Easy to monitor the drug efficacy on tumor growth by examining visible size	May not fully reproduce human cancer tissue because of poor stroma involvement
	Heterotopic model		Efficacy data in this model may not accurately correlate with clinical outcomes in some cases
	Orthotopic model	Account for tissue microenvironment for cancer cells where originated or metastasized	Requires relatively complicated methods for transplantation
	Autocthonous model	Reproduce carcinogenesis-associated events such as host inflammation	Difficult to monitor tumor growth over time
	Carcinogen-induced model		Requires complicated methods and expects potential variability among individual animals
			Difficulties in preparing a sufficient number of mice and relatively time-consuming
			Difficult to maintain mouse with multiple mutant alleles
			May not accurately reproduce human cancer types
			Challenges for using drug efficacy evaluation (tumor latency, time for tumor formation etc.)
			Accuracy of the model in its clinical relevance has been questioned in some cases
			Clear restriction in availability and utility
Human cancer model	Transplantation model	Ability for testing human cell lines in relevant tumor types or with genetic backgrounds	
	Cell line	Ability for testing clinical patient-derived tumor tissues	
	PDX		
Spontaneous dog cancer model	Naturally occurring canine cancer	Share many characteristics with human malignancies	Difficulties in preparing a sufficient number of dogs

Summary of the characteristics of preclinical animal models and their potential advantages and disadvantages for use in oncology drug development. GEM, gene-engineered mouse; PDX, patient-derived xenograft.

**Table 2. Characters of genetically engineered mouse models**

Mutation type	Conventional mutation		Conditional mutation	
	NA	Viral (e.g. adex-Cre)	Tissue-specific (e.g. GFAP-Cre, FABP-Cre)	Induced (e.g. R26-CreERT2, Tyr-CreERT2)
Mutation induction	NA	Viral (e.g. adex-Cre)	Tissue-specific (e.g. GFAP-Cre, FABP-Cre)	Induced (e.g. R26-CreERT2, Tyr-CreERT2)
Generation of embryonic lethal knockout animals	Not available	Available	Available	Available
Tissue specificity	Uncontrollable Tumors generated are not necessarily present in the same tissues as those in humans	Induce tissue-specific/local mutation Tumors can be generated in the same tissues as those in humans	Induce selective mutation at a cellular level Reproduce cancer initiating cells	Inducible selective mutation at a tissue or cellular level
Time specificity	No	Controllable	Promoter-dependent Uncontrollable	Promoter context Controllable
Induction process	NA	Extremely complicated Tissue limitation	NA	Required (but not complicated)
Induction efficiency	Excellent	Low	Promoter-dependent Relatively high	Promoter-dependent Difficult to achieve high efficiency
Homogeneity of tumors	Relatively consistent	High variability Skill-dependent	Low variability	Low variability Skill-dependent
Acquisition of the number of mice	Easy	Difficult	Easy	Manageable (but requires induction process)
Maintenance of mouse strains	Generally easy (dependent on target genes; difficult in the case of tumor generation in heterozygous mice)	Easy	Complicated to maintain animals having multiple mutant alleles	Complicated to maintain animals having multiple mutant alleles

This table summarizes the advantages and potential problems in various types of genetically engineered mouse models for use in preclinical studies of oncology drugs. NA, not applicable.

therapeutic drugs for cancer in companion animals is being actively pursued in the USA and Europe. Considering the pathology of cancer in large breed dogs seems to be similar to those in humans,<sup>(68)</sup> the utility of spontaneous cancer in large breed dogs for testing new oncology drugs has already been initiated in the USA and Europe.<sup>(69)</sup> In Japan, the leading cause of death in dogs is also cancer with a mortality of 54% (“The Ten Leading Causes of Death in Dogs and Cats” reported by the Animal Insurance System Japan Animal Club), which is much higher than the mortality rate of other diseases such as heart disease (17%). Given these circumstances, studies for developing methods for the diagnosis and treatment of cancer in dogs have been actively initiated. Based on the results of these studies, the Japanese Society of Clinical Veterinary Medicine have been discussing the significance of cancer models using companion animals in non-clinical studies for developing oncology drugs as well as preparing for the establishment of relevant administrative and management systems for its application.

### Evaluation of Oncology Drugs that Directly Target Cancer Cells

The efforts of oncology drug development originally concentrated on the production of drugs that directly target the proliferation or metabolic properties of cancer cells. Along with discovery of oncogenic driver genes, development of molecular targeted drugs has been highlighted, which directly pinpoint signal transduction pathways involving those driver genes, as well as the protein degradation systems, epigenome, and metabolic systems of cancer cells. As molecular targeted drugs, ty-

rosine kinase inhibitors (TKI), multi-targeted kinase inhibitors (MTKI), and drugs that target molecular mechanisms for cell cycle regulation and others have been successfully developed. Although the classical anticancer chemotherapeutic drugs also show cytotoxicity by attacking specific intracellular molecules, the term “molecular targeted drug” in this report is defined as a drug that has been developed through primary identification of a molecule or a signaling pathway as a therapeutic target, which is highly activated or deregulated in cancer cells. Table 4 summarizes the pros and cons for evaluating molecular targeted drugs in non-clinical cancer models. The results produced by the use of these models have been included in the application of new drugs; the models believed to be essential.

**Tyrosine kinase inhibitors and other kinase inhibitors.** Tyrosine kinase inhibitors include epidermal growth factor receptor inhibitors (gefitinib, erlotinib, lapatinib, and afatinib), human epidermal growth factor receptor 2 inhibitors (lapatinib and afatinib), anaplastic lymphoma kinase inhibitors (crizotinib, ceritinib, and alectinib), BCR-ABL inhibitors (imatinib, dasatinib, nilotinib, ponatinib, and bosutinib), a KIT inhibitor (imatinib), SRC inhibitors (dasatinib and bosutinib), a JAK inhibitor (ruxolitinib), a Bruton’s tyrosine kinase inhibitor (ibrutinib), and a dual kinase MEK inhibitor (trametinib). There are several other kinase inhibitors, including BRAF inhibitors (vemurafenib and dabrafenib), a phosphatidylinositol-3 kinase inhibitor (idelalisib), and mammalian target of rapamycin inhibitors (temsirolimus and everolimus). In addition, drugs that target p38, AKT, p70S6 kinase, insulin-like growth factor 1 receptor, platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), MET, ROS 1, and RET are currently being developed. For evaluating the effica-

Table 3. Mouse models corresponding to genetic mutations in human cancers

Human disease		Mouse model			
Cancer type	Mutated gene	Mutated gene	Mutation type	Mutation induction	Tumor produced
Medulloblastoma	<i>RB1</i>	<i>Rb1/Tp53</i>	Conditional KO/conditional KO	GFAP-Cre	Medulloblastoma <sup>(18)</sup>
		<i>Rb1/Bmi1</i>	Conditional KO/conditional activation	GFAP-Cre	Medulloblastoma <sup>(19)</sup>
	<i>PTCH1</i>	<i>Ptch1</i>	Conditional KO	math1-cre/ GFAP-Cre	Medulloblastoma <sup>(20)</sup>
Gorlin syndrome	<i>PTCH1</i>	<i>Ptch1</i>	Conventional		Medulloblastoma, rhabdomyosarcoma <sup>(21)</sup>
Pituitary gland tumor	<i>RB1</i>	<i>Rb1</i>	Conventional KO	Pomc-Flp	Pituitary gland tumor <sup>(22,23)</sup>
		<i>Rb1</i>	Conditional KO		Pituitary gland tumor <sup>(24)</sup>
Lung cancer	<i>KRAS</i>	<i>Kras</i>	Conventional KO (sporadic activation)	Adex-Cre	Lung cancer <sup>(25)</sup>
	<i>BRAF</i>	<i>Braf</i>	Conditional activation		Lung cancer <sup>(26,27)</sup>
	<i>RB1</i>	<i>Rb1/Tp53/Pten</i>	Conditional KO/conditional KO/ conditional KO		CGRP-CreER
Breast cancer	<i>EML4-ALK</i>	<i>EML4-ALK</i>	Conventional activation (SPC promoter)	Tet system	Lung cancer <sup>(29)</sup>
	<i>EML4-ALK</i>	<i>EML4-ALK</i>	Conditional activation		Lung cancer <sup>(30)</sup>
	<i>KIF5B-RET</i>	<i>KIF5B-RET</i>	Conventional activation (SPC promoter)		Lung cancer <sup>(31)</sup>
	<i>EZR-ROS1</i>	<i>EZR-ROS1</i>	Conventional activation (SPC promoter)		Lung cancer <sup>(32)</sup>
	<i>PIK3CA</i>	<i>Pik3ca</i>	Conditional activation	MMTV-Cre	Breast cancer <sup>(33)</sup>
	<i>TRP53</i>	<i>Pik3ca/Tp53</i>	Conditional activation/conditional KO	MMTV-Cre	Breast cancer, leukemia <sup>(34)</sup>
	<i>PTEN</i>	<i>Pten</i>	Conditional KO (stromal fibroblast)	Fsp-Cre	Breast cancer <sup>(35)</sup>
Hereditary breast cancer	<i>ERBB2</i>	<i>ErbB2</i>	Conventional activation (MMTV promoter)	MMTV-Cre	Breast cancer <sup>(36,37)</sup>
		<i>ErbB2/Pten</i>	Conditional activation/conventional KO		Breast cancer <sup>(38)</sup>
	<i>RB1</i>	<i>Rb1/Tp53</i>	Conditional KO/conditional KO	MMTV-Cre	Breast cancer <sup>(39)</sup>
	<i>BRCA1</i>	<i>Brca1/Tp53</i>	Conditional KO/conventional KO	BLG-Cre	Breast cancer <sup>(40)</sup>
		<i>Brca1/Chk2</i>	Conditional KO/conventional KO	Wap-Cre	Breast cancer <sup>(41)</sup>
Colorectal cancer	<i>BRCA2</i>	<i>Brca2/Tp53</i>	Conditional KO/conventional KO	K14-Cre	Breast cancer, skin tumor <sup>(42)</sup>
	<i>APC</i>	<i>Apc/Kras</i>	Conditional KO/conditional activation	Adex-Cre	Colorectal cancer <sup>(43)</sup>
	<i>KRAS</i>	<i>Apc/Kras</i>	Conditional KO/conditional activation	Fapbl-Cre	Colorectal cancer <sup>(44)</sup>
Familial adenomatous polyposis	<i>PTEN</i>	<i>Apc/Pten</i>	Conditional KO/conditional KO	Cyp1a1- CreERT2	Tumor of the digestive tract <sup>(45)</sup>
	<i>Smad4</i>	<i>Apc/Smad4</i>	Conventional KO/conventional KO		Tumor of the digestive tract <sup>(46)</sup>
	<i>APC</i>	<i>Apc</i>	Conventional KO		Tumor of the digestive tract <sup>(47-49)</sup>
		<i>Apc</i>	Conditional KO	Adex-Cre	Tumor of the digestive tract, <sup>(50)</sup> liver cancer <sup>(51)</sup>
Hereditary non-polyposis colorectal cancer	<i>MSH3</i>	<i>Msh3</i>	Conventional KO		Lymphoma <sup>(52)</sup>
	<i>MSH6</i>	<i>Msh6</i>	Conventional KO		Lymphoma, <sup>(52)</sup> tumor of the digestive tract, skin cancer, uterine cancer <sup>(53)</sup>
		<i>Msh3/Msh6</i>	Conventional KO		Lymphoma, <sup>(52)</sup> tumor of the digestive tract, <sup>(54)</sup> skin tumor <sup>(53)</sup>
Cowden syndrome	<i>PTEN</i>	<i>Pten</i>	Conventional KO		Tumor of the digestive tract, lymphoma, adrenal tumor, breast cancer, prostate cancer <sup>(55,56)</sup>
Pancreatic cancer	<i>KRAS</i>	<i>Kras/Tp53</i>	Conditional activation/conditional KO	pdx1-cre	Pancreatic cancer <sup>(57)</sup>
		<i>Kras/Tgfbr2</i>	Conditional activation/conditional KO	Ptf1a-cre	Pancreatic cancer <sup>(58)</sup>
		<i>Kras/Pten</i>	Conditional activation/conditional KO	pdx1-cre	Pancreatic cancer <sup>(59)</sup>
Endometrial cancer	<i>PTEN</i>	<i>Pten/Mig6</i>	Conditional KO/conditional KO	PR-Cre	Endometrial cancer <sup>(60)</sup>
		<i>Pten/Tp53</i>	Conditional KO/conditional KO	PR-Cre	Endometrial cancer <sup>(61)</sup>
Ovarian cancer	<i>KRAS</i>	<i>Kras/Pten</i>	Conditional activation/conditional KO	Adex-Cre	Ovarian cancer <sup>(62)</sup>
	<i>APC</i>	<i>Apc</i>	Conditional KO	Pgr-Cre	Ovarian cancer <sup>(63)</sup>
Prostate cancer	<i>BRCA2</i>	<i>Brca2/Tp53</i>	Conditional KO/conventional KO	K18-Cre	Ovarian cancer <sup>(64)</sup>
	<i>BRCA2</i>	<i>Brca2/Tp53</i>	Conditional KO/conventional KO	Pbsn-Cre	Prostate cancer <sup>(65)</sup>

Table 3 (Continued)

Human disease		Mouse model			
Cancer type	Mutated gene	Mutated gene	Mutation type	Mutation induction	Tumor produced
Skin tumor	<i>BRAF</i>	<i>Braf</i>	Conditional activation	Tyr-CreERT2	Malignant melanoma <sup>(66)</sup>
		<i>Braf/Pten</i>	Conditional activation/conditional KO	Tyr-CreERT2	Malignant melanoma <sup>(67)</sup>
	<i>PTCH1</i>	<i>Ptch1</i>	Conditional KO	R26-CreERT2	Basal cell tumor <sup>(20)</sup>

Mouse models reproducing generative tissues and mutations found in human cancer. While many other scientifically excellent mouse models for human cancers have been generated, the table preferentially lists those harboring relatively simple mutant alleles suitable for preclinical studies. It should be noted some mouse models do not completely recapitulate pathologies of human cancer.

cies of those kinase inhibitors, transplantation models with target (mutant) gene-positive cancer cells or GEM models driven by target (mutant) genes have been generally used. In general, cancer cells that have potent driver gene mutations (“gain-of-function” mutations) show a high degree of so-called oncogene addiction, and therefore it would be relatively easy to predict or evaluate the drug response *in vivo*. These non-clinical cancer models are also useful for evaluating pharmacodynamics of the drugs by monitoring the phosphorylation status of the target molecules, their downstream factors, or both. Meanwhile, it should also be noted that established cancer cell lines may have altered their phenotypes and characters compared with the original cancers during *in vitro* culture, whereas genetically engineered cell lines may not be able to accurately replicate the etiology of the relevant clinical cancer types.

**Multitargeted kinase inhibitors.** Multitargeted kinase inhibitors include a RAF/vascular endothelial growth factor receptor-2 (VEGFR-2)/PDGFR- $\beta$  inhibitor (sorafenib), a VEGFR2/PDGFR- $\beta$ /KIT/FLT-3 inhibitor (sunitinib), a VEGFR/KIT/PDGFR inhibitor (pazopanib), a RET/VEGFR2/EGFR inhibitor (vandetanib), a VEGF/PDGF inhibitor (axitinib), a VEGFR/RET/KIT/PDGFR/RAF inhibitor (regorafenib), a MET/RET/VEGFR/KIT/FLT-3/TIE-2/TRKB/AXL inhibitor (cabozantinib), and a VEGFR/FGFR/PDGFR/SRC/LCK/LYN/FLT-3 inhibitor (nintedanib). Similarly to TKIs, the efficacy of MTKIs can be evaluated in non-clinical cancer models. However, MTKIs target multiple kinases and it is generally difficult to prepare genetically engineered cell lines that reproduce the pathology of the target cancers. In the case of MTKIs that target angiogenic factors, such as VEGFR, FGFR, and PDGFR, accurate prediction of *in vitro* efficacy would be difficult: pazopanib, for example, does not necessarily show a direct antiproliferative effect on many cancer cell lines *in vitro*, but it significantly inhibits tumor growth *in vivo* by blocking angiogenesis.<sup>(74)</sup> Also, because MTKIs could have multiple modes of action, establishment of the proof-of-concept at the pharmacodynamic level in non-clinical cancer models might require a complex procedure.

**Targeting cell cycle.** Palbociclib inhibits cyclin-dependent kinases 4 and 6 (CDK4 and CDK6), which are involved in cell cycle control. Furthermore, drugs targeting various cell cycle regulators, such as WEE1, cell division cycle 7, checkpoint kinase 1 and 2, ATR, Aurora, PLK, and mitotic kinesins, are under clinical development. Efficacies of these drugs can be evaluated using relevant cancer cell lines that have abnormalities in the target molecules or their regulators (e.g. CCND1/CDK6 amplification or CDKN2 deletion/mutation) in transplantation models.

**Targeting protein degradation systems.** Protein degradation systems have been recognized as an emerging therapeutic

target for particular types of cancer. While several target molecules have been described in this category, proteasome inhibitors, such as bortezomib and carfilzomib, have been developed most extensively and approved as anticancer drugs. Meanwhile, other molecular targets include the NEDD8-activating enzyme, the ubiquitin-activating enzyme, and stress proteins that are involved in protein folding, such as heat shock protein 90 and glucose-regulated protein 78. Given that the preferential efficacies of proteasome inhibitors against multiple myeloma have been well established, transplantation models with multiple myeloma cell lines could be applicable for evaluating the efficacy of the drugs in this category. However, there are several potential issues and limitations for predicting the clinical efficacy of these drugs from non-clinical cancer models: detailed mechanisms for the action of the drugs and predictive biomarkers for the drug responses are rather elusive, and cancer types that are susceptible to the anticancer effects of the drugs in non-clinical studies may not be consistent with those in the clinical settings. Therefore, the latest knowledge from basic research and clinical phase I studies on various cancer types should be taken into consideration for additional indication of the drugs.

**Targeting genomes and epigenomes.** The anticancer efficacies of drugs that target cancer epigenomes, such as DNA methyltransferase inhibitors (azacytidine and decitabine) and histone deacetylase (HDAC) inhibitors (vorinostat, panobinostat, romidepsin, and belinostat), have been shown *in vivo*, although the cancer types against which the drugs are effective differ between the non-clinical studies and clinical practice in some cases.<sup>(84)</sup> As these drugs affect many target sites in a genome-wide manner, detailed mechanisms and predictive biomarkers for the drug response often remain elusive. Drugs targeting the genomic repair systems include poly(ADP-ribose) polymerase (PARP) inhibitors, such as olaparib. Because there is a synthetic lethal relationship between PARP and tumor suppressors, BRCA1 and 2, it would be relatively easy to predict the therapeutic efficacy of PARP inhibitors by using transplant models of cell lines with BRCA1 or 2 deficiency.<sup>(85,86)</sup> Besides BRCA1/2, it has been also postulated that there are many synthetic lethal factors with PARP inhibition. However, the clinical validity of those candidates has not been fully established. However, it should be also noted that synthetic lethality confirmed in the non-clinical studies (e.g. effect of a PARP inhibitor on EWS-FLI1-positive Ewing’s sarcoma)<sup>(87,89)</sup> could be sometimes abolished by the formerly applied therapies in the clinical settings.

**Targeting cancer cell metabolisms.** Metabolic enzymes favored by cancer cells, such as isocitrate dehydrogenases 1/2 (IDH1/2) and fatty acid synthase, are potential targets for cancer therapy. For IDH1/2 inhibitors, transplant models of IDH1

Table 4. Evaluation of drugs directly targeting cancer cells

Classification (type of inhibitors)	Target molecule	Evaluation methods (drug efficacy study)	Characteristics	Problems
Tyrosine kinases	EGFR, HER2, ALK, BCR-ABL, KIT, SRC, JAK, BTK, IGF1R, PDGFR, FGFR, MET, ROS1, RET	(i) Transplantation models of target (mutant) gene positive cancer cells Cancer cell lines with target (mutant) genes <sup>(70)</sup> Alternative cell lines into which target (mutant) genes are transfected <sup>(71)</sup> (e.g. Ba/F3) (ii) GEM models <sup>(29)</sup>	Can predict/evaluate drug efficacy in the model with potent driver gene activities and oncogene addiction <sup>(72)</sup> Can generate resistant cells as negative control Can establish proof-of-concept pharmacodynamically by evaluating autophosphorylation of target kinases or phosphorylation of downstream factors Can predict/evaluate drug efficacy in the model with potent driver gene activities <sup>(31)</sup>	(i) Cancer cell lines may change their phenotypes during the process of their establishment due to selective pressure and stresses (ii) Alternative cell lines may not accurately replicate the etiology of the relevant cancer types
Kinases (multi-targeted)	RAF, VEGFR-2, PDGFR- $\beta$ , KIT, FLT-3, RET, EGFR, MET, RET, TIE-2, TRKB, AXL, SRC, LCK, LYN	The same as (i) and (ii) above <sup>(31)</sup> For anti-angiogenic agents, Matrigel plug assay could be used <sup>(73)</sup>		In addition to (i) and (ii) above: It is difficult to generate alternative cell lines reproducing the pathology of target cancers by genetic engineering when the drug acts on multiple kinases in the target cancer cells <i>In vitro</i> cell growth assays do not reflect the antiangiogenic action <i>in vivo</i> <sup>(74)</sup> May require complicated pharmacodynamic analyses due to the presence of multiple targets In addition to (i) and (ii) above: (iii) It is difficult to achieve sufficient drug response in some cancer types including colorectal cancer with less potent driver activities, in which other coexisting (i.e. not mutually exclusive) driver pathways contribute to tumor proliferation <sup>(77)</sup> The same as (i), (ii), and (iii) above
MAPK pathway	MEK, BRAF, p38	Cancer cell lines with mutations in the target pathway of interest (target molecule or upstream target) or transplantation animal models with alternative cell lines generated by genetic engineering <sup>(75,76)</sup> GEM models <sup>(27)</sup>	Can predict/evaluate drug efficacy in the model with potent driver gene activities <sup>(77)</sup> Can establish proof-of-concept pharmacodynamically by evaluating phosphorylation of downstream factors	
PI3K/mTOR pathway	PI3K, mTOR, AKT, p70S6K	Cancer cell lines with mutations in the target pathway of interest (target molecule or upstream target) or transplantation animal models with alternative cell lines generated by genetic engineering <sup>(78)</sup> GEM models <sup>(33)</sup>	Can predict/evaluate drug efficacy in the model with potent driver gene activities <sup>(79)</sup> Can establish proof-of-concept pharmacodynamically by evaluating phosphorylation of downstream factors	
Cell cycle	CDK4/6, WEE1, CDC7, CHK1, CHK2, ATR, Aurora, PLK, mitotic kinesins	Cancer cell lines with mutations in the target pathway of interest (target molecule or upstream target) or transplantation animal models with alternative cell lines generated by genetic engineering <sup>(80)</sup>	Drug efficacy may be achieved in cancer cell lines with an abnormality as shown in the left-hand column	The same as (i), (ii), and (iii) above

Table 4 (Continued)

Classification (type of inhibitors)	Target molecule	Evaluation methods (drug efficacy study)	Characteristics	Problems
Protein degradation system	Proteasome, related target molecules (NEDD8-activating enzyme, ubiquitin-activating enzyme, HSP90, GRP78)	Allograft/xenograft models of multiple myeloma cell lines <sup>(81)</sup>	Can predict/evaluate drug efficacy with multiple myeloma cell lines used in the studies of previously developed drugs	In addition to (i) above: (iv) Cancer types for which drugs are effective in preclinical studies may not be consistent with those in clinic
Genome/epigenome	DNMT, related target molecules (histone methyltransferase, histone demethylase)	Allograft/xenograft models of MDS cell lines <sup>(82)</sup> MDS models generated by implanting MDS cell lines into genetically engineered NSG mice <sup>(83)</sup>	MDS mouse models replicate the pathology more accurately than other transplantation animal models	In addition to (i) and (iv) above: Due to a very small number of available cell lines, clinical relevance of the model may be limited (v) Due to the genome-wide distribution of target sites, detailed mechanisms of action and predictive biomarkers for the drug response remain unclear
	HDAC	Allograft/xenograft models of colorectal/prostate/lung cancer cell lines <sup>(84)</sup>	Drug efficacy may be achieved in some cancer types in addition to those shown in the left-hand column	The same as (i), (iv), and (v) above Cutaneous T-cell lymphoma and peripheral T-cell lymphoma are currently approved for HDAC inhibitors
	PARP1/PARP2, related target molecules (DNA-dependent protein kinase, telomerase)	Allograft/xenograft models of cancer cell lines with <i>BRCA1</i> or <i>BRCA2</i> (tumor suppressor gene) mutation or inactivation <sup>(85,86)</sup>	Can predict/evaluate drug efficacy by using cancer cell lines with <i>BRCA1/2</i> deficiency: there is a synthetic lethal relationship between <i>PARP1/2</i> and <i>BRCA1/2</i>	The same as (i) and (iv) above In addition to <i>BRCA1/2</i> , substantial numbers of synthetic lethal factors are reported, (however, most of them are described only at a basic research level and the clinical relevance has not been fully established) Synthetic lethality may be diminished by pretreatment in the clinical cases even if preclinically confirmed <sup>(87)</sup>
Metabolic systems	IDH1/IDH2 (mutant-type), Fatty acid synthase	Xenograft models of IDH1 (R132)/IDH2 (R172) mutant-positive AML or glioma cell lines <sup>(88)</sup>	Can predict/evaluate drug efficacy by examining the presence of mutation Pharmacodynamic study can be carried out by monitoring mutation-specific metabolites (oncometabolites) <sup>(88)</sup> Drugs targeting molecules that produce no oncometabolites may be effective to a wider range of cancer types	If the target produces no oncometabolites, mechanisms of action or predictive biomarkers for the drug response may not be available and it may be difficult to design evidence-based studies to evaluate the drug response

This table classifies the target molecules of approved/investigational drugs used in Japan, overseas, or both and lists representative non-clinical evaluation methods of these drugs. Due to their usefulness and usability, evaluation results have been used for publication data of original papers and oncology drug application dossiers for approval. Meanwhile, it should be noted that these technologies have technical limitations and contain a number of limitations/problems attributable to the properties or unclarified factors of target molecules and diseases. ALK, anaplastic lymphoma kinase; BTK, Bruton's tyrosine kinase; CDC7, cell division cycle 7; CHK, checkpoint kinase; DMNT, DNA methyltransferase; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; GRP, glucose-regulated protein; HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2; HSP, heat shock protein; IDH, isocitrate dehydrogenase; IGF1R, insulin-like growth factor 1 receptor; MDS, myelodysplastic syndromes; mTOR, mammalian target of rapamycin; PARP, poly(ADP-ribose) polymerase; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol-3 kinase; VEGFR, vascular endothelial growth factor receptor.

(R132) or IDH2(R172) mutation-positive AML and glioma cell lines are useful for predicting drug efficacies.<sup>(88)</sup> The pharmacodynamics of these drugs can be evaluated by monitoring the mutation-specific metabolite (oncometabolite), 2-

hydroxyglutaric acid. However, if the target molecule does not produce a characteristic oncometabolite, one may expect a broader spectrum of anticancer efficacies of the inhibitors. In that case, however, it may be relatively difficult to evaluate

**Table 5. Evaluations of drugs targeting angiogenesis and tumor stroma**

Classification	Target	Evaluation method (drug efficacy study)	Characteristics	Problems
Targeting angiogenesis	Angiogenic factors (ligands) e.g. VEGF antibody	(i) Mouse cancer models (ii) Human cancer models (iii) Angiogenesis models (e.g. Matrigel plug assay, CAM assay, hollow fiber assay)	Evaluate in mouse/human cancer transplantation models with drugs and targets exhibit cross-reactivity between species Mechanisms of action can be examined depending on phenotypes of target molecule deficiency in GEM models	(i) Mouse transplantation models, GEM models (ii) Human cancer models: Cross-reactivity of the target molecule in mice should be considered (iii) Angiogenesis models: Consider the cross-reactivity of the drug between species. Generally difficult to evaluate drug efficacy in chemical carcinogen-induced models
	Receptors/receptor signals e.g. TKI (VEGFRs)	As above, (i), (ii), and (iii)	(i) Mouse transplantation models (ii) Human cancer models (cell line transplantation, PDX): The effect of the drug on mouse angiogenesis can be evaluated Mechanisms of action can be examined depending on phenotypes of target molecule deficiency in GEM models	As above, (i) and (ii).
	Production of angiogenesis factors e.g. mTOR inhibitor	As above, (i), (ii), and (iii)	(i) Mouse transplantation models (ii) Human cancer models (cell line transplantation, PDX): The effect of the drug on mouse angiogenesis can be evaluated. Mechanisms of action can be examined depending on phenotypes of target molecule deficiency in GEM models.	(i) Mouse transplantation models, GEM models: Consider the cross-reactivity of the drug between species. (ii) Human cancer models: Cross-reactivity of the target molecule in mice should be considered (iii) Angiogenesis models: Difficult to evaluate drug efficacy due to the lack of angiogenesis factor production
Targeting tumor stroma	Drug resistance/sensitivity, growth/metastasis, inflammation	(i) Mouse/human cancer transplantation model (s.c. transplantation models, orthotopic transplantation/metastasis models), cancer cell-stromal cell co-transplantation models (ii) GEM models	(i) Evaluate in mouse/human cancer transplantation models with drugs and targets exhibit cross-reactivity between species (ii) Mechanisms of action can be examined depending on phenotypes of target molecule deficiency in GEM models	(i) Transplantation models: Consider the cross-reactivity of the drug (mouse) or target (human). Human cancer s.c. transplantation models: Difficult to evaluate drug efficacy due to insufficient involvement of microenvironments (ii) GEM models: Cross-reactivity of the target molecule in mice should be considered. Generally difficult to evaluate drug efficacy in chemical carcinogen-induced models

Animal (mainly mouse) models used for the evaluation of oncology drugs targeting angiogenesis and tumor stroma are classified in this table. As the efficacy of these drugs depends on cancer–host interactions or host factors, consideration should be given to the cross-reactivity of therapeutic drugs and/or their target molecules between species (mainly between humans and mice). CAM, chick chorioallantoic membrane; GEM, gene-engineered mouse; mTOR, mammalian target of rapamycin; PDX, patient-derived xenograft; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

the efficacy of the drugs because the mechanism of action and predictive biomarkers would remain unclear.

### Targeting Cancer Cell–Host Interactions

The importance of microenvironments on the growth, progression, and therapeutic resistance of cancer cells has been drawn much attention. Such tumor microenvironments have been known to support cancer cell proliferation directly or indirectly through interactions between surrounding stroma cells. In general, it is relatively difficult to carry out an appropriate *in vivo* efficacy test for drugs targeting interactions between cancer cell and host microenvironment in non-clinical cancer models.

**Targeting angiogenesis.** It has been widely recognized that generation of new blood vessels into tumor (angiogenesis) is a critical step for cancer cells to be adequately supplied nutrition and oxygen, therefore, it is assumed that tumors are unable to grow progressively without angiogenesis. There are also several relevant studies suggesting that angiogenesis is involved in not only cancer cell proliferation but also cancer cell progression, including metastases to distant organs. As represented by VEGF inhibitors (bevacizumab), drugs targeting angiogenesis may not exert direct antitumor effects on cancer cells, however, should inhibit the activity of various angiogenic factors that mainly affect vascular endothelial cells for generating new

blood vessels. Consequently, non-clinical evaluation of the efficacy of drugs targeting angiogenesis can be greatly affected by host factors in experimental animals; therefore, it is critical to use appropriate models for drug evaluation, as summarized in Table 5.

For carrying out appropriate *in vivo* tests for drugs targeting angiogenesis, it is very important to consider whether cancer cell lines or patient-derived samples produce angiogenic factors for targeting and, moreover, their cross-reactivity in non-clinical cancer models. It is also relevant for other angiogenesis models such as the Matrigel plug assay, chick chorioallantoic membrane assay, or hollow fiber assay.

**Targeting cancer stroma.** Diverse cellular components of tumor stroma (e.g. fibroblasts, mesenchymal cells, and inflammatory cells) and extracellular matrices (e.g. fibronectin, collagen, laminin, and proteoglycan) have been shown to be involved in cancer cell proliferation and progression. Although tumor stroma is expected to be an attractive therapeutic target, the development of drugs targeting cancer stroma is still in the early stages.

Similar to those targeting angiogenesis, non-clinical evaluation of drugs targeting tumor stroma should be greatly affected by host factors. In immune-compromised mice (e.g. nude, SCID, NOD/SCID, and NOG) often used for transplantation models of human cancer cells display a range of different

**Table 6. Evaluations of drugs targeting host immune response**

Model	Outline	Characteristics	Problems
Allograft model	Syngeneic (mainly mouse) cancer cell lines implanted into s.c. as heterotopic transplantation models, or implanted into original tissues/organs in orthotopic transplantation models, or injected into tail vein as metastasis models Use of cell lines with ectopic expression of model antigens (e.g. OVA, <sup>(90,91)</sup> HA, <sup>(92)</sup> CEA <sup>(93)</sup> ) or cell lines known with their immunogenicity (e.g. B16 melanoma, <sup>(94)</sup> Meth A, <sup>(95)</sup> colon 26 <sup>(96)</sup> )	Immune responses against cancer cells can be monitored over time and the mechanism of action can be tested Tumor antigen-specific immune responses can be evaluated where antigens have been specified Orthotopic transplantation models and metastasis models may be better for analyzing tumor-infiltrating lymphocytes considering the organ microenvironment of cancer cells.	Heterotopic transplantation models may not immunologically completely reproduce human cancer tissues due to insufficient tumor stroma Orthotopic/metastasis models require technical skills and are generally difficult for quantitative monitoring of tumor growth.
Carcinogen-induced mouse model	Mouse models developing tumors by challenging with carcinogenic substances (e.g. MCA, AOM/DSS, DMBA/TPA), or external stimuli such as UV, or inducing genetic abnormalities (e.g. p53 deficiency, transduction of SV40T antigen, APC deficiency)	Immune response during the carcinogenic process can be evaluated The clinical cancer pathology is closely represented.	Requires complicated procedure and poses difficulty in maintaining mouse strains Longer experimental period Difficult to evaluate antigen-specific immune response due to the lack of defined tumor antigens with some exceptions
Xenograft (human cancer) model (includes PDX)	Xenograft with human cell lines or patient-derived tumor tissues into immune-compromised mice (e.g. nude mice, SCID mice, NOG mice).	Antitumor activities can be analyzed by using human (cancer patients') immune cells.	Limitation for analyzing immune responses due to its incompetence of the intact immune system Application of humanized mice engrafted with human immune cells clearly requires further investigation

Animal (mainly mouse) models used for evaluating drugs targeting host immune response are classified in this table. As the efficacy of cancer immunotherapy depends on the host's immune system, concurrent use of multiple models should also be considered. In such a case, it is necessary to devise optimal combinations of models to be used, taking into account the potential limitations/problems of each model presented in the table as advantages or disadvantages. AOM, azoxymethane; APC, Adenomatous polyposis coli; CEA, carcinoembryonic antigen; DMBA, 7,12-dimethylbenz(a)anthracene; DSS, Dextran sulfate sodium; HA, hemagglutinin; MCA, 3-Methylcholanthrene; OVA, ovalbumin; PDX, patient-derived xenograft; TPA, 12-O-Tetradecanoyl-phorbol-13-acetate.

immunological environments. Even in these immune-compromised animals, myeloid compartment and mesenchymal cells are known as relatively normal, therefore the efficacy of drugs targeting those stromal cells may be evaluated even in animal models if the target shows cross-reactivity between species.

**Targeting host immune responses.** The immune system has been regarded as an important constituent of the tumor microenvironment. Many series of studies have been undertaken to understand the regulatory mechanisms by which cancer cells control, either positively or negatively, hosts' immune responses. Recent clinical successes of immune checkpoint inhibitors, such as anti-CTLA-4 mAbs (ipilimumab and tremelimumab) and anti-PD-1 mAbs (nivolumab and pembrolizumab) highlight targeting hosts' immune responses against cancer cells as a promising target for drug development.

Obviously, drugs targeting hosts' immune responses should be tested in the appropriate non-clinical cancer models in which the targets are involved in the immune responses against cancer cells, for elucidating the mechanisms of action and predicting potential side-effects. In general, it is ideal to test the importance of drug targets or potential drug candidates in different experimental models (multiple cell lines, different mouse strains). Considering there should be a limitation for predicting cancer types to which the drug shows clinical benefit by testing only in non-clinical models, the results of phase I clinical studies need to be carefully considered. For testing drug candidates in which certain HLA haplotypes are required

to show antitumor effects (e.g. cancer vaccine therapy), an application of humanized mice may be worth considering as non-clinical models. In Table 6, we summarize pros and cons of non-clinical models for testing drugs targeting hosts' immune responses.

### Evaluation of Oncology Drugs Based on New Concepts

Along with gaining our knowledge with the biological characteristics of cancer, there are several new approaches to develop oncology drugs, such as targeting cancer stem cells.

**Targeting cancer stem cells.** The concept of cancer stem cells was originally introduced in hematological malignancies and further extended to solid cancers such as breast cancer and brain tumors.<sup>(97)</sup> Cancer stem cells have been characterized by their self-renewal potential, multidirectional differentiation potential, and niche dependence, similar to other stem cells, in addition to their highly tumorigenic potential. Furthermore, cancer stem cells have been known for their resistance to conventional chemotherapy or radiotherapy; therefore, they may be an emerging target for drug development. In Table 7, we summarize the current methods for testing drugs targeting cancer stem cells in non-clinical evaluations.

**Targeting other novel concepts or methods.** In Table 8, we summarize the current status of oncology drug development targeting new concepts other than cancer stem cells, or novel methods for developing new oncology drugs. Non-clinical evaluation of some of those oncology drugs targeting novel

**Table 7. Evaluation of drugs targeting cancer stem cells**

Evaluation method	Outline	Characteristics	Problems
Spheroid formation potential	Culture a single non-adherent cell in the presence of specific growth factors (without serum) to test the capability of forming spheroids	Evaluation can be made using cultured cells, and the dose- and time-dependence can be quantitatively measured	General cytotoxicity of drugs mislead as positive without testing on normal tissue stem cells
Cell surface marker	Measuring the frequency of CD44 high/CD24 low fraction, known as cancer stem cells in breast cancer by flow cytometry	Cytotoxic drugs can be tested by comparing effect on cancer stem cell fraction and others	Surface markers for cancer stem cell fractions differ depending on cancer types
ALDH	ALDH activities positively correlate to chemoresistance and stemness in breast cancer, gastrointestinal tract cancer, and hematological tumors	Established methods for measuring activity by flow cytometry	Not all ALDH-positive cells are cancer stem cells
Xenograft models with human cancer stem cells in immune-compromised mouse	Human cancer stem cells transplanted into immune-compromised mice for testing drug efficacy on tumor formation /growth	Evaluating the inhibitory effect of drugs on tumor formation or growth and cancer stem cell frequency within tumor tissue (assessed based on surface markers, ALDH, and spheroid formation potential)	Not applicable for testing drugs targeting immune responses or microenvironments
Syngeneic mouse models with mouse cancer stem cells	Mouse cancer stem cells transplanted into syngeneic mice for testing drug efficacy on tumor formation /growth	Evaluating the inhibitory effect of drugs on tumor formation or growth and cancer stem cell frequency within tumor tissue (assessed based on surface markers, ALDH, and spheroid formation potential) Applicable for testing drugs targeting immune responses or microenvironments	Efficacy may need to be confirmed in models using human cancer stem cells
Genetically engineered animal models	Testing drugs targeting cancer stem cells using genetically engineered mice, rats, or zebrafish to develop tumors	Ideal models closely resembles an autochthonous tumor	Evaluation requires a prolonged time period because of late onset of cancer compared with transplantation models

This table lists commonly used methods to evaluate cancer stem cell functions. ALDH, aldehyde dehydrogenase.

**Table 8. Emerging new concepts in oncology drug development**

Example	Outline	Problems	International comparison (e.g. clinical study information)
Nucleic acid medicine	Chemically synthesized oligonucleotide	Need to consider appropriate DDS for tumor targeting, efficiency for cellular uptake, organ accumulation such as liver	Japan: Phase I Overseas: Phase I–III (sponsored by OncoGenex Pharmaceuticals Inc., etc.)
Oncolytic virus	Modified viruses reacting specifically against tumors	Requirement for support system of clinical studies/international joint research, review system, guideline establishment, and research funds	Japan: Phase I–II Overseas: Approved (China); phase I–III (USA and Europe)
Cell therapy	Regenerative therapy using iPS cells or immune cell therapy	Tumor development risk Accumulation of evidence for therapeutic efficacies	Japan: Phase I–II Overseas: Approved (USA); phase I–III
Nanotechnology-based drugs	Application to DDS; treatment using microscopic particles (embolization therapy)	Safety concerns by using nano-materials Tumor-specific delivery	Japan: Phase I–III Overseas: Approved; phase I–III
Companion diagnostic drugs	Diagnostic drugs to evaluate the efficacy and safety of specific drugs	Not fully available for all pharmaceutical products Appropriate review system Not fully clear for applying medical service payment system	Japan: <i>ALK</i> fusion gene, <i>KRAS</i> gene mutations, etc. Overseas: <i>BRAF</i> gene mutations, and many others
Hyperthermia	Delivery of antineoplastic agents to a tumor by heat	Safety concerns by using nano-materials	Japan: Phase I–II Overseas: Phase I–III
Imaging-based therapy	Specific labeling of cancer cells; effective for evaluation of treatment effects	Not applicable to all cancer types Requirement for efficacy/safety verification	Japan: Under development Overseas: Practical use in assessment of the effect of cell transplantation therapy
Cancer cell line panel†	Assessment of mechanisms of action of candidate molecules using a set of diverse cell types	Limited number of cell lines (potential expansion) Distinct nature from actual human tumor samples	Japan: Panel of human cancer cell lines (JFCR39) Overseas: NCI-60 cell lines (NCI/NIH, USA); ATCC tumor cell panels (USA); Oncolines™ cancer cell line panel contains 66 cancer cell lines (NTRC, Netherlands)

This table exclusively presents oncology drugs that are being or about to be investigated in Japan and overseas based on new concepts.

†Although “Cancer cell line panel” cannot be classified as a therapeutic drug, it is presented here as an assay that is extensively used in the development of new therapeutic drugs. DDS, drug delivery system; iPS, induced pluripotent stem cells.

concepts may require approaches that are different from those used for the evaluation of conventional oncology drugs.

A deeper understanding of the biological characteristics of cancer is leading to the development of novel oncology drugs based on new concepts such as “cancer stem cells” in addition to the developmental targets presented in earlier sections.

### Concluding Remarks

This review summarizes present non-clinical investigations by listing the common methods currently used for the development of oncology drugs as extensively as possible. Their types, profiles, and problems are briefly described. Characteristics of a variety of animal models, which provide indispensable information to formulate clinical research and clinical trials, are summarized according to each category of oncology drug. Experimental models obtain the proof of evidence at the molecular, cellular, and tissue levels, and unique oncology drugs are also covered. It is hoped that this review provides information to undertake regulatory science relevant to the development of oncology drugs.

Studies with cancer models, including animal experiments, *ex vivo* studies, and *in vitro* studies, are essential technology in cancer biology and have contributed to the development and evaluation of oncology drugs. Particularly, cancer cell lines derived from humans and experimental animals have been

used for decades as indispensable tools for the biological understanding of cancer and for the development of oncology drugs. Properties of cancer cells represented by a cell have been changing cell line, it was discovered that the accumulation of multiple abnormalities in genes causes cancer and that the properties of individual cancer cell lines depend not only on their organ origins but also on the types of abnormal genes. Growing knowledge on cancer as a disease has led to the understanding that interactions between cancer and host cells and the regulatory molecules play critical roles. The growth of tumors strongly depends on tissue microenvironments and immunological milieu that are difficult to reproduce *in vitro*. As shown in this review, a substantial number of models reflecting these various aspects of cancer–host interactions have been developed in the past decade. These models have significantly contributed to the expansion of the range of non-clinical studies and their role, in the exploration, development, and clinical investigation of oncology drugs have become indispensable.

The diversity and the degree of engagement in genetic changes in the initiation of cancer cell growth and progression are widely accepted. The roles of host cells, tissue, and the immune system also vary depending on the type, properties, and the stage of individual tumors are also becoming clear than before. Therefore, the methods used to select and use oncology drugs should continuously be revised based on the

advance in understanding of cancer. As stated earlier in this review, models established for the biological understanding of cancer have proven to be useful as tools for non-clinical investigations. When developing a new drug that is in the same class as those for which efficacy and safety information was already acquired from clinical studies, it is also useful to select non-clinical models based on the clinical information. Collectively, it will become increasingly important to design, to select, and to use appropriate non-clinical models in order to design clinical research and trials. Investigations with these models should be effective in interpreting the results of such investigations and to re-evaluate the effects of oncology drugs used in clinical practice. It is strongly hoped that non-clinical investigation will continuously be successfully used for the

development, approval, and proper use of oncology drugs, which accelerate drug development.

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