medical and scientific failures, negative results are rarely analyzed and reported in the scientific literature.

1-2. Analyses of chemotherapeutic failures

. These therapeutic failures are attributed to many causes, as described below.

1-2-1. Indiscriminate drug distribution to normal tissues and tumors, with no tumor-selective drug delivery. The first reason for these chemotherapeutic failures is that conventional low-MW cancer drugs, most of which are cytotoxic, are distributed indiscriminately throughout the entire body, in various organs and tissues, with little tumor-selective drug accumulation. Therefore, systemic toxicity, including nausea, anorexia, bone marrow suppression, hematotoxicity, peripheral neurotoxicity, alopecia, diarrhea, and kidney and liver toxicity, frequently occurs, and increasing the drug doses is not possible because the doses used are already approaching the maximum tolerable dose. In addition to these adverse effects, immunological suppression, which is often observed in these patients, provides cancers with an environment for easier growth and progression.

1-2-2. Genetic diversity or heterogeneity. The second reason for chemotherapeutic failures is that cancer cells *in vivo* mutate continuously during

carcinogenesis and during the so-called progression stage, which may last from 10 to 30 years, as seen in gastric cancer and hepatoma, both of which involve chronic infection. In such a setting, an inflammatory reaction induces reactive oxygen species (ROS), including reactive nitrogen species,[2)-6)] that causes mutations in lesions that are frequently irreversible or that the DNA repair systems cannot fix the damage because they may be also damaged.

Recent advances in cancer genomics have indicated that the average patient with lung cancer, for example, would have 100-200 mutant cancer cells. Each patient with esophageal or colon cancer would have approximately 50-100 mutant cancer cells.[7)-9)] Therefore, the most recent molecular target drugs, which aim at a specific, unique molecular target present in a given cancer, would have about a 1% chance of therapeutic success. Monoclonal antibody drugs (and cancer vaccines), which use as molecular targets antigenic epitopes, such as protein tyrosine kinases or their receptors including vascular angiogenesis factor (vascular endothelial growth factor, or VEGF) and epidermal growth factor (EGF) receptors, respectively, can also manifest mutations. Therefore, cancer cells can escape molecular recognition via mutations that will nullify targets of the molecular drugs.[7)-9)] Furthermore, in many cases, antibodies, for instance those to VEGF or EGF receptors, do not eradicate cancer cells but frequently

make them quiescent.[10)-13)] We have experienced that once the antibody or the receptor inhibitor, is blocked or inactivated, cancer cells resume growth again.

Many related clinical studies have shown limited success with these treatments despite approval of regulatory authorities.[1),13)-15)] In view of these data, scientists at the National Institute for Health Care and Excellence (NICE), in the United Kingdom, and others in academia and the media have expressed concerns about the cost-effectiveness of these drugs[13)-15)] (see the discussion in Section 1-2-4 below).

1-2-3. Immunotherapy: still not decisive weaponry against cancer. The initial concept of immunotherapy was based on the principle that newly emerged cancer cells would possess one or more new antigenic potentials that would provoke an effective immunological reaction if the host had normal immunological potency. Clearly, cancer patients lose the immunological capacity to combat the cancer cells, possibly because of aging of hosts (cancer patients). The cancer cells may therefore escape from the immunological host response and surveillance, and a host's defense cells may not see cancer cells as immunologically different, or cancer cells may destroy the tumor-attacking immune cells.

Cytotoxic T-cells offer potential hope in this immunotherapy strategy, in which

antibody-like molecules (called T-cell receptors) develop on the cell surface. Comparable to soluble antibodies, T-cell receptors can recognize a diverse repertoire of antigenic epitopes of target (cancer) cells.

Investigations of numerous immunotherapies along this line were performed with tumor-bearing mouse models. One method utilized *in vitro* externally activated T-lymphocytes that were infused into the host. In such an experimental setting, this treatment was effective when the number of effector cells (cancer-killing immune cells) (E) was 30- to 50-fold higher than the number of target tumor cells (T). That is, an E/T ratio of 30 or more is needed. However, when tumors in humans weigh 5-10 g, about 150-300 g of activated cytotoxic effector cells must be infused. This target is almost impossible to achieve, and curing patients is therefore difficult. Although this information is known, many clinical treatments using this method are performed in Japan, even though the National Health Insurance has not approved the treatment method. However, standard protocols for bladder cancer worldwide still utilize the well-known evidence of immune activation by bacterial cell components (*e.g.*, BCG). In this context, activation of innate immunity such as macrophages or natural killer cells may be worthwhile.

1-2-4. Issues related to the stability of liposomal and micellar drugs in relation to the enhanced permeability and retention (EPR) effect and tumor accumulation. The third reason for chemotherapeutic failures concerns active-drug encapsulated liposomal or micellar nanoparticles. This nanotechnology-based therapy with nanomedicines has been the focus of great attention in the past 2-3 decades (*e.g.*, Ref. 16). Particles that had the poorest consideration on the clearance by macrophages or phagocytes demonstrated failed treatments because they were quickly removed from the circulation by phagocytic cells or reticuloendothelial cells. However, a current method of attaching biocompatible polymers such as polyethylene glycol (PEG) to the surface of the particles can protect them against phagocytosis or the immunological surveillance system.

Another issue is the rigid, sturdy structure of these particulate drugs, such as Doxil®, which consists of pegylated liposomes containing doxorubicin (DOX). When drug-encapsulated liposomes or nanoparticles are not stable enough, a drug may leak out of the drug-encapsulated-particles during circulation, or the particles may burst in a short time, and the effect becomes similar to that of the parental low-MW drug (see Fig. 1b). Therefore, design of macromolecular drugs consisting of a stable, biocompatible complex would lead to effective drug accumulation in solid tumor tissue by virtue of the EPR effect, which is discussed later in Section 2. However, when the drug release was in fact too slow, because of the stability of the study liposome, it would result in poor drug action; the consequence being an effective concentration of the only *stable liposome itself in the tumor tissue,* but a poor concentration of the active drug principle in the tumor, and thus a poor clinical response (such as with Doxil®).

In the case of unstable micellar drug complexes, however, physical disruption

during circulation may occur as micelles burst during intravenous (i.v.) injection. Consequently, the plasma concentration of these micelles quickly drops, with no EPR effect seen (Fig. 1b). Figure 1 presents hypothetical examples of the plasma pharmacokinetics of different low-MW drugs versus polymer-conjugates or nanodrugs.

Both a low-MW free drug such as tetrahydropyranyl doxorubicin (THP) (Fig. 1a) and an unstable micellar complex of DOX and a copolymer (Fig. 1b) were cleared too rapidly from blood circulation. The plasma stability of a styrene-co-maleic acid (SMA)-polymer DOX conjugate (Fig. 1c) and an SMA-polymer THP conjugate (Fig. 1d) and release of the drug (DOX) from the conjugate (Fig. 1c) were significantly better than those of the complex of DOX and a copolymer (Fig. 1b), but the release was too rapid to have an improved EPR effect. In comparison, the SMA-polymer THP conjugate (Fig. 1d) had the best release rate and plasma and tumor concentrations, with the result being better therapeutic efficacy because of the improved EPR effect.

The micellar drug of Fig. 1b is an example of chemotherapeutic failure at an early clinical stage (NK-911), in that the stability of this particular micellar drug was insufficient so that the micelles burst too quickly; about 50% released within 1 hour after i.v. injection, and thus no benefit from the EPR effect could be obtained which requires a circulation time of a drug for several hours or longer. In this context, the biocompatible polymer (hydroxypropylmethacrylamide) [HPMA], MW～3K, conjugated to DOX (PK-1) also failed to show the EPR effect, as seen in Fig. 1b. This point is critical for biocompatible macromolecular drugs, which must have a high plasma level for a very long time, such as several hours to a day or more, which may be possible with macromolecular drugs of MW > 50Da.

A few recent reports commented about unsatisfactory results concerning

tumor-selective accumulation of nanomedicines as based on the EPR effect[17),18)] (as discussed later in Section 2). With regard to these data, the bursting of the micellar structure or release of the encapsulated drugs during circulation is critically important. In the experiments cited here, the polymer carrier was covalently linked with a fluorescent probe so as to follow the *in vivo* biodistribution via fluorescence. The micelles are non-covalently encapsulated the candidate drug (tritiated paclitaxel). The *in vivo* distribution study revealed, after i.v. injection of the fluorescent micelles, a clear tumor-selective EPR effect. However, when the researchers analyzed the accumulation by radioactivity count of the drug (paclitaxel) in the tumor, they found a different result: the accumulation of the drug in the tumor was somewhat less than 1%, which is similar to that of the free form of the low-MW drug paclitaxel (Taxol®). These researchers thus concluded that no EPR effect occurred for the nanoparticle drug. Their experiments did not analyze, for example, spontaneous drug release from the micelles in the culture medium, drug release in the presence of NaCl, and that in blood plasma. However, non-covalently encapsulated low-MW drugs (such as paclitaxel) would have leaked out rapidly from the micelles in the presence of blood, whereas the covalently linked fluorophore to the polymer would have remained as macromolecules and exhibited the EPR effect. Thus, careful interpretation of these results is required, and experiments should be designed to avoid such artefacts.

1-2-5. Problems in cancer drug screening and evaluation. Drug-screening models that use implanted tumors may not be equivalent to spontaneous tumors in found in clinical situations. The most important point in drug development relates to a drug's

effectiveness: it should produce beneficial results against both solid primary and metastatic tumors. Traditional primary drug screening has been performed with mouse peritoneal leukemia L1210 and P388 models, with drugs being administered intraperitoneally (i.p.) and tumors being implanted i.p. In this system, a given drug may be readily accessible to tumor cells in the peritoneal cavity. Pharmacological properties such as plasma level, tissue distribution, inactivation in the liver and renal elimination, and access to the neovasculature in the tumors are of secondary importance; the immediate drug action in the i.p. compartment determines the efficacy of the drug. Thus, any cytotoxic drug candidates may demonstrate good effects in the peritoneal cavity, but these effects may not apply to solid tumors, which have unique vascular and tissue properties (*e.g.*, neoangiogenesis, permeability, hypoxic characteristics). Therefore, EPR effect-based targeting of drugs to tumors does not exist in the i.p./i.p. system.

The second problem concerns the mouse model itself, which is usually a syngeneic and/or human xenograft model. However, no syngeneic humans exist except for identical twins. In the syngeneic mouse model, the tumor (xenobiotic) has good immunological compatibility with the host, and thus a host reaction to a xenobiotic tumor would not occur, because the tumor would be immunologically inert. Host mice

for human xenograft tumors also do not produce immunological reactions. This model may work well for HIV/AIDS patients but not for cancer patients in general. In addition, the time frame for rodents and humans is quite different. The life spans of mice and rats are extremely different from the human life span. Experimental mouse tumors grow rapidly, *e.g.*, 5×10^6 cells implanted in a mouse reach a palpable size in about a week, whereas human tumors usually take months to years to reach a noticeable size. The optimal time scale for the slow release of cancer drugs that would be effective in humans would therefore be quite different from that in mice.

In addition, anatomical sites used for implanting tumors are frequently located in skin or muscle, not in orthotopic tissues. Consequently, one can argue against the validity of a vascular similarity of those sites compared with the original organs. That is, renal cancer or hepatoma implanted in muscle tissue cannot have the same vascular network as the network in the kidney or in the liver, respectively. In this respect, autochthonous models or chemically induced breast, colon, or liver cancer may serve as much better models or more realistic tumors. Furthermore, metastatic tumor models are rarely used for drug screening.

I also want to emphasize that the most common endpoint in the murine screening system is prolongation of survival (life span) compared with survival of a control group

receiving no drugs. All mice would eventually die, but cures with tested drugs or conventional anticancer agents are seldom seen, particularly with metastatic tumors. Therefore, an endpoint of a cure rate with a different time frame, *e.g.*, more than 100 days or a significantly longer time period, should be used. Evaluation of cancer drugs should consider the cure rates and no disease recurrence as seen in development of antibiotics for infectious diseases. In addition, the so-called therapeutic window should be large enough for improved safety and therapeutic efficacy, and polymer-conjugated drugs usually provide a lower toxicity compared with the parent drug, *e.g.*, Doxil® versus DOX.

1-2-6. Problems in photodynamic therapy. PDT has been known for more than a century. Indeed, N.R. Finsen received the Nobel Prize in Medicine and Physiology in 1903 for his novel phototherapy of dermal tuberculosis. PDT was expanded to cancer treatment half a century ago as the use of the helium-neon (HeNe) laser, which emits a monochromatic light at 635 nm. However, PDT requires a photosensitizer with a given range of wavelength for photoexcitation (such as seen xenon or some other source) to generate singlet oxygen, *i.e.*, an oxygen radical (an ROS), which is the cancer-killing principle in PDT. Current PDT methods fail to fulfill the basic principle of