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New approaches to drug discovery and development: a mechanism-based approach to pharmaceutical research and its application to BNP7787, a novel chemoprotective agent

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Abstract Any approach applied to drug discovery and development by the medical community and pharmaceutical industry has a direct impact on the future availability of improved, novel, and curative therapies for patients with cancer. By definition, drug discovery is a complex learning process whereby research efforts are directed toward uncovering and assimilating new knowledge to create and develop a drug for the purpose of providing benefit to a defined patient population. Accordingly, a highly desirable technology or approach to drug discovery should facilitate both effective learning and the application of newly discovered observations that can be exploited for therapeutic benefit. However, some believe that drug discovery is largely accomplished by serendipity and therefore appropriately addressed by screening a large number of compounds. Clearly, this approach has not generated an abundance of new drugs for cancer patients and suggests that a tangibly different approach in drug discovery is warranted. We employ an alternative approach to drug discovery, which is based on the elucidation and exploitation of biological, pharmacological, and biochemical mechanisms that have not been previously recognized or fully understood. Mechanism-based drug discovery involves the combined application of physics-based computer simulations and laboratory experimentation. There is increasing evidence that agreement between simulations based on the laws of physics and experimental observations results in a higher probability that such observations are more accurate and better understood as compared with either approach used alone. Physics-based computer simulation applied to drug discovery is now considered by experts in the field to be one of the ultimate methodologies for drug discovery. However, the ability to perform truly comprehensive physics-based molecular simulations remains limited by several factors, including the enormous computer-processing power that is required to perform the formidable mathematical operations and data processing (e.g. memory bandwidth, data storage and retrieval). Another major consideration is the development of software that can generate an appropriate and increasingly complex physical representation of the atomic arrangements of biological systems. During the past 17 years, we have made tremendous progress in addressing some of these obstacles by developing and optimizing physics-based computer programs for the purpose of obtaining increasingly accurate and precise information and by improving the speed of computation. To perform physics-based simulations that involve complex systems of biological and pharmaceutical interest, we have developed methods that enable us to exceed Moore's law. This has been accomplished by parallel processing as well as other methods that have enabled us to study more complex and relevant molecular systems of interest. This paper provides an overview of our approach to drug discovery and describes a novel drug, currently in clinical development, which has directly resulted from the application of this approach.

Keywords Drug discovery · Mechanism-based · Cancer drugs · Chemoprotectant (BNP7787, Tavocept) · Platinum and taxane drugs

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Introduction

Future improvements in the treatment of patients with cancer, including the development of curative or more

efficacious and less toxic treatments, will be the product of pharmaceutical discovery and development efforts that target entirely new mechanisms or exploit known mechanisms in previously unrecognized and better ways. Any approach applied to drug discovery and development by the medical community and pharmaceutical industry has a direct impact on the future availability of improved, novel, and curative therapies for patients with cancer. Drug discovery and development is a high-risk endeavor: current estimates for the average cost and time required to develop a single new drug are US$ 897 million and up to 15 years from the point of initial chemical synthesis of a new compound [25]. Therefore any technology or improved organization of research and development activities that can facilitate more effective learning, application of newly discovered observations, or knowledge that can be exploited for therapeutic benefit are highly desirable.

In this paper, we delineate the drug discovery and development process as a complex learning and problem-solving endeavor whereby research efforts are directed at uncovering and assimilating new knowledge to create and develop safe and effective new drugs that will ultimately provide benefit in a defined patient population. As a part of this, we describe the importance and the perspective of applying a mechanism-based approach to drug discovery; this approach involves the combined application of physics-based computer simulations and laboratory experimentation. We present the view that drug discovery approaches involving serendipity or the assumption that evaluating larger numbers of compounds as a means to increase the probability of success should not be expected to play a material role by themselves in successful drug discovery and development programs. It is increasingly clear that agreement between physics-based molecular simulations and experimental observations can result in a substantial reduction in the number of experimental trials in the discovery process, thereby improving productivity and an earlier and more in-depth understanding of a drug's mechanism(s) of action.

Outcomes using a drug discovery approach based on serendipity and a belief in probability and game theory applied to large numbers of compounds

It is not possible to predict an outcome for what is not already known or mentally conceived, such as a hypothesis or a new chemical composition. When knowledge of possible outcomes has been conceived or is known (e.g. head or tail of an evenly balanced coin or a physiological response to a pharmacological intervention), then the probability of such outcomes can be estimated based on the depth and breadth of knowledge of the operating principles of the subject matter. In the case of cancer treatment, to develop safe and effective new drugs, it appears that there is a great deal that remains unknown. Although we know more today about the pathogenesis, pathophysiology, biological, and biochemical features of cancer, there appears to be a large gap in our understanding of how to cure selectively and effectively the most common and deadly types of cancer (e.g. advanced cancers of the lung, breast, colon, prostate, pancreas, melanoma, bladder, and brain). Furthermore, we have not fully characterized all the mechanism(s) of action of the currently approved drugs used to treat cancer; this is largely because we cannot say at this time that the biology of normal and malignant cells has been fully characterized and the molecular interactions of currently available drugs and all the biochemical effects that such drugs have on normal versus malignant cells have been elucidated. The problem in part is that much of the research has focused on the identification of new compounds and new and unvalidated targets, but it appears that we do not have a comprehensive understanding of the underlying mechanisms that can be exploited for patient benefit either as improvements in drug safety, efficacy, delivery, specificity, formulation, or combinations of these prerequisite endpoints.

It is not difficult to find statements in the media or scientific literature that drug discovery *has been* or *can be* accomplished largely by serendipity, or by beating the odds, or the notion that pharmaceutical discovery productivity can be effectively enhanced by screening large numbers of chemical compounds from various sources (thereby increasing the number of opportunities for serendipity). The public promotion of these concepts has resulted in higher expectations for faster and more prolific new drug discoveries. The notion is that technology, serendipity, and increasing the number of compounds evaluated will lead to better medicines faster by simply playing an odds game on increasingly larger scales. Notwithstanding the distinction between creative learning processes and probability game theory, during the past 20 years of observation in this field, it appears increasingly clear that such approaches have not resulted in the generation of a plethora of approved new drugs in general, and particularly in oncology.

If drug discovery is an exercise in probability or odds that can be solved by screening large numbers of compounds, this reasoning begs the question: can one predict the discovery of a drug? If probability game theory applies to drug discovery, then the calculated probability and the actual outcome should be strongly correlated, and therefore the outcome will be predictable. Thus far, there does not appear to be any correlated evidence supporting the notion that the number of compounds screened bears any relationship to the probability of successful drug approval. Today, there is convincing evidence that drug discovery and development productivity are not meeting these high expectations based on the fact that the discovery and development pipelines in the pharmaceutical industry are dwindling, the costs of drug development are increasing exponentially, and yet the number of drug approvals is holding at or near the

same level during this period. This combination of unsuccessful events is leading to mergers and acquisitions of pharmaceutical companies because they are unable to produce new products more predictably and reliably. This is a notable outcome to consider because the pharmaceutical industry has the highest research and development expenditures of any industry in the world, exceeding US$ 30 billion, and this research and development spending has doubled approximately every 5 years during the past 15 years [17]. In recent years, there have been fewer new drug approvals, with no evidence of greater success as measured by the number of drug approvals, or by the number of drugs approved divided by the total number of discovery research areas. This outcome is not a failure of these technologies; it may be the result of an overly optimistic expectation that was created for these technologies and a generalized failure to consider that drug discovery is a complex learning process and that research and development should be organized in a manner that takes these factors into consideration.

It is important to appreciate that drug discovery involves complex multidisciplinary problem solving, learning and creativity, and by definition, drug discovery itself is an unpredictable endeavor. Drug discovery is a difficult creative learning process involving problem solving and the application of new knowledge to pursue the creation and/or use of a specific arrangement of atomic matter (a drug) that when administered in a prescribed manner results in a significant medical benefit to patients. Discovering a new drug that is safe and effective is not an easy task because of the interdisciplinary requirements for this entire learning and application process. It is interesting to consider that penicillin, methotrexate, 5-fluorouracil, platinum, camptothecin, paclitaxel, and many other chemotherapy drugs, were discovered by individuals who made astute observations of outcomes that were previously unrecognized, and notably in every case, they followed up on these new observations with additional research that enabled new knowledge to be obtained and applied, and this eventually resulted in the successful development of these agents as approved drugs [6, 7, 14, 19, 20, 27]. The Bar Harbor incident involving nitrogen mustard was clearly an accidental exposure, and the observations that were subsequently made led to the discovery and development of better more active alkylating agents for the purpose of treating patients with cancer [10]. It is important to recognize that essentially none of these discoveries were made by luck or by accident; they were the result of prepared and observant investigators who continued to pursue research relevant to the new observation (the discovery), which resulted in the eventual realization of the potential utility of the new observations. It is an arduous task to develop new agents into approved safe and effective drugs; in all of these and other cases, the span of many years elapsed before the original discovery was ultimately an approved drug.

Application of physics-based molecular simulations and computational chemistry to drug discovery: integration of classical physics, statistical mechanics, and ab initio quantum mechanics

In the 16th century, Newton conceived and wrote a coherent description of the properties of motion, gravity, and force that defined classical physics. Classical physics-based molecular simulations are based on Newton's law of motion ($F = ma$), wherein Newton's equations of motion ($F = d^2v/dt^2$) are integrated as $F = [x(t + dt) = x(t) + x(t)dt + x(t)dt^2/2]$; these terms correspond to atomic position, velocity, and acceleration of each individual atom in the system into a force-field equation that represents the total energy and the chemical state of a system composed of defined bonds, angles, dihedrals, nonbonded atoms (Van der Waals forces), and Coulombic (electrostatic) terms for atomic charges and the dielectric constant of any surrounding solvent for an ensemble arrangement of atoms that define a molecular system of motion.

In our physics-based molecular simulations, we utilize Newton's laws of motion in combination with perturbation of chemical states (e.g. determining differences in physicochemical and thermodynamic properties between molecule A and molecule B) and statistical mechanical methods to characterize the chemical, thermodynamic, and conformational behavior and properties of biological and molecular systems. These types of simulations can provide information regarding the equilibrium conformational and thermodynamic behavior of molecular systems, the net differences in Gibbs free energy between two systems, as well as the Gibbs free energy of binding of different species of drugs, for example, to a protein or nucleic acid target. These types of simulations pose certain computational problems, not the least of which is that the complexity and computational time required scales as N^2 (where N is the number of particles or atoms in a system); thus the computational requirements for a system with 1000 atoms (a small molecular system) versus 100,000 atoms (large protein plus water) varies by one million pairwise calculations at each step versus 10 billion pairwise energy calculations per step, respectively. We have addressed this problem by developing highly optimized parallel vector-processing computer programs along with new algorithms (e.g. enabling larger time steps with numerical stability) that allow us to gain an enormous speed-up in the computational time, roughly $N^2/\log N$. This capability has enabled us to perform large-scale, time-dependent simulations on three-dimensional protein folding, protein and nucleic acid structure determination, and corresponding drug interactions involving biological systems of more than 150,000 atoms.

In 1926, Erwin Schrödinger conceived and formulated equations that accurately represented the energetic behavior and probability distribution of electrons and atomic nuclei, thereby defining the field of quantum

mechanics. This field of physics is one of the most fundamentally important discoveries of the last century because it can be applied to any research involving arrangements of atomic matter. It has taken approximately 50 years to develop increasingly sophisticated computer programs that implement the actual physics and mathematical formalisms as optimized executable codes that can be applied to drug discovery. In 1998, John Pople received the Nobel Prize for Chemistry for his work in developing sophisticated and novel physics-based algorithms for computational chemistry that are based on the application of ab initio (literally meaning 'first principles') quantum mechanics. Pople is the originator of the mathematical applications of the ab initio quantum mechanical Gaussian computer program that is in widespread use today. As a product of Pople's and others' efforts, ab initio quantum computational chemistry has become a recognized field of research for drug discovery. Most companies do not fully utilize this methodology due to the enormous computational requirements. It is estimated that 5% of all computer simulations performed in the pharmaceutical industry employ this methodology.

The accuracy and precision of quantum mechanics can be applied to chemical, structural, electronic, vibrational, and thermodynamic systems and simulations, and it has the greatest accuracy (how well it matches with experimental outcomes) and precision (how reproducible the results are) of all physics-based computational methods. It is widely used in the materials science and chemical engineering fields to discover and optimize new products that are composed of small molecules. At the same time, quantum physics-based simulations are the most formidable to perform due to the exponentially increasing and numerically intensive nature of quantum mechanics. At the simplest level of quantum mechanics application, the Hartree-Fock level or single electronic configuration of a molecular system, the computational problem scales as N^4, where N is the number of single electron mathematically defined basis functions that define the system. To improve conformational, thermodynamic, electronic, and chemical accuracy, much higher orders of quantum mechanical methods must be applied; however, these scale at orders of N^6 to as high as N^{12}. Even the most effective parallel processing cannot overcome these higher-order computational requirements for larger molecular systems. However, there have been recent developments that have reduced the computational execution speed scaling by 100-fold. We routinely perform more than 50% of our total workload of computer simulations using this methodology.

Advances in computer-processing power needed for physics-based simulations for drug discovery

Physics-based computer simulation applied to drug discovery is now considered to be the ultimate simulation methodology by experts in the field. However, the ability to perform truly comprehensive physics-based molecular simulations has been limited by several factors, including the computer-processing power that is required to perform the formidable number of complex mathematical operations and data processing (e.g. memory bandwidth, data storage, and retrieval). Another limitation is the need for more optimized and complex molecular simulation software that can generate and perform time-dependent property simulations on the increasingly complex three-dimensional physical representation of the atomic arrangements of biological systems. Many research groups, including ours, have made substantial progress in addressing some of these obstacles by developing and optimizing physics-based computer programs for the purpose of obtaining increasingly accurate and precise information as well as to improve the speed of program execution.

Physics-based simulations of drug interactions with biological targets are referred to as a 'Grand Challenge Problem' in high-performance computing; this designation is specific for the most formidable computing problems. Gordon Moore in 1965 predicted that computer-processing power would double every 18 months; this prediction is known as Moore's law and has been consistently achieved from that time to the present and is predicted to hold for the foreseeable future. To perform physics-based simulations that involve complex systems of biological and pharmaceutical interest, we have had to develop methods that enable a physics-based computer simulation greatly to exceed Moore's law. This has been accomplished by parallel and vector processing as well as other methods that have enabled us to study more complex and relevant molecular systems of interest. Today, it is possible to achieve 10^{12} (trillions or teraFlops) decimal arithmetic operations per second on integrated computing systems, such as the Cray X-1 (Cray Inc., Seattle, Wash.). To study large regions (e.g. the nucleus) of the cell, we estimate that it will require systems capable of performing approximately 10^{15} (petaFlops) decimal arithmetic operations per second. The application of parallel vector systems that will be capable of supporting 500 teraFlop to petaFlop capability will have a major impact on the life sciences by allowing physics-based molecular simulations of cellular processes, drug-target interactions, assessment of potential favorable and unfavorable metabolism of drugs, simulations of time-dependent protein behavior and folding, genomic instability/misrepair/frameshifting, drug delivery via passive diffusion, drug formulation optimization, and numerous other important physical, chemical, biochemical, pharmacological, and biological properties. We estimate that this objective will be achieved within the next 5 years, which will have an impact on the research community's ability to engineer better drugs for patients with cancer.

What is mechanism-based drug discovery?

A basic tenet for improved success in drug discovery is that, in contrast to serendipity or "random" screening of

large numbers of compounds, the substantial majority of successful pharmaceutical discovery research programs involve a priori learning and insights regarding key molecular, chemical, pharmaceutical, biological, and biochemical properties and behavior of target systems, and their interrelationships to understand exploitable mechanism(s) of action for the target and potential drug. This is a difficult problem-solving and creative endeavor requiring a highly coordinated multidisciplinary approach to learning and exploiting known and/or new mechanisms with greater accuracy, precision, and the attempt to understand operative mechanisms in greater depth.

To discover a new drug, one must increase the probability that the desired outcome will be observed as well as the probability of determining whether a drug candidate's properties are a true and correct observation. This approach is aimed at reducing the risk of compound failure at later stages of development. This probability determination is increased as a consequence of applying new knowledge from physics-based simulations that are complementary to experimental observation, because drug discovery is a relatively intractable multiple-variable problem involving chemical properties and the biological, pharmacological, and biochemical behavior of the drug with its target. This is challenging because there are many variables in this problem-solving exercise that are known, and there are yet many unknown biological, chemical, or structural variables that are difficult or impossible at present to define in a computer program or by experiment. However, because learning is involved, one can modify such simulations or experiments in accordance with new observations that result from laboratory testing or simulation.

Mechanism-based drug discovery involves the concurrent investigation of five major areas that are essential to drug discovery: (1) characterization of the biological target and optimal drug–target interactions; (2) drug safety–toxicity arising as a consequence of unfavorable chemical features and metabolic propensity; (3) improving drug delivery by preventing inactivation by metabolism and pharmacological properties; (4) creating a stable and safe drug formulation by multivariable excipient optimization based on physicochemical properties and excipient safety; and (5) refinement of the synthetic chemistry operations for achieving an optimized chemical, electronic, and structural/conformational molecule that fulfills all required objectives of areas 1–4.

Mechanism-based drug discovery is based on the elucidation and exploitation of biological, chemical, pharmacological, and biochemical mechanisms, not previously recognized or fully understood, which can potentially advance the treatment of cancer by pharmaceutical intervention. This approach to drug discovery is based on a fully integrated concurrent application of physics-based computer simulations and laboratory experimentation. In the event that there is agreement between physics-based simulations and the experimental observations, the probability that such observations are true is greater than if one relies on either approach alone. In addition to facilitating the discovery process as a learning effort, this approach is aimed at reducing the probability of failure and enhancing the development process.

Mechanism-based drug discovery research is based on the following principle: if a series of molecular simulations of the properties (time-dependent, conformational, and thermodynamic) of a biological target, chemical transformations, stability and interactions, or drug–target interactions of interest that are correctly applied to the laws of physics (classical and quantum mechanics and statistical mechanics and thermodynamics) are in agreement with a series of experimental observations of the molecular systems of interest, the corresponding probability that such observations are true and correct is greatly increased. This principle is based on the recognition that there is an inherent weakness in performing physics-based simulations in the absence of experimental observations as well as performing experiments without corroboration of physics-based models. In either instance a hypothesis cannot be fully evaluated in accordance with what can be experimentally observed or measured and quantified based on the laws of physics. The physics-based component of problem solving and hypothesis testing requires the application of high-performance computing due to the otherwise intractable mathematical composition.

Mechanism-based drug discovery is an integrated multidisciplinary system-based approach to drug discovery that focuses on obtaining new knowledge and applying it to elucidating the underlying mechanisms that are operative to achieve therapeutic benefit. One additional feature of this approach is fairly certain: to our knowledge, physics-based computer simulations are never performed by accident nor are the outcomes achieved by luck. Another expectation for this research model is that no technological approach alone or in combination with others is likely to improve the number of discoveries per unit of time. The most reasonable expectation is that there will be greater depth of understanding and a better ability to exploit new mechanisms with greater precision, less cost, and a higher success-to-failure ratio. For example, if the clinical success-to-failure ratio can be increased from 1 in 10 to 1 in 5 compounds, this would be an important advance.

Results from the application of the mechanism-based drug discovery approach

Mechanism-based drug discovery has allowed us to identify and exploit several previously unrecognized mechanisms and to identify new drugs that are aimed at achieving greater safety and efficacy for cancer treatment. This application can be illustrated by one of our drugs that is currently in phase III clinical trials. This is one of several examples of drugs discovered using a

mechanism-based approach that are now undergoing development.

BNP7787: a novel chemoprotective agent for prevention of common and serious toxicities associated with chemotherapy

An important and common problem of chemotherapy today is the drug-induced toxicities that frequently prohibit effective dose intensity or dose density of chemotherapy. Today, some of these toxicities, such as anemia or neutropenia, nausea and vomiting, can be effectively prevented or mitigated by drugs that have been developed and approved for these purposes. However, there remain no effective or approved therapies for several common and serious chemotherapy-induced toxicities, such as peripheral neurotoxicity resulting from treatment with platinum (cisplatin, oxaliplatin, carboplatin), taxane (paclitaxel, docetaxel, and others), vinca alkaloids, thalidomide, ara-G, ara-C, epothilones, oxazaphosphorines (chloracetaldehyde), and other agents. Nephrotoxicity due to cisplatin has also been problematic, particularly with the resurgence of interest in the use of cisplatin over carboplatin due to significantly improved survival rates in patients with advanced non-small-cell lung cancer [18].

BNP7787 (disodium-2,2′-dithio-bis ethane sulfonate, Tavocept) is currently undergoing worldwide clinical development to prevent or mitigate common and clinically important toxicities associated with taxane- and platinum-type chemotherapeutic agents. Our initial focus was to discover a novel chemoprotective agent that would prevent or mitigate common and serious toxicities associated with platinum-based chemotherapy, including neurotoxicity and nephrotoxicity. We later expanded this focus to include other drugs that cause neurotoxicity, due to the discovery of several general mechanisms that underlie neurotoxicity as well as those that are responsible for other forms of clinically important toxicities that are currently unaddressed. For drug discovery projects, it is important at the outset to specify the desired drug properties and outcomes to facilitate the formulation of testable hypotheses, and while it is a difficult exercise, it is an essential practice. Therefore we defined discovery and development goals for an ideal chemoprotective agent at the outset of this project that were followed and refined during the process. These goals included the following:

- Prevention, mitigation, or delay of serious common chemotherapy toxicities and treatment delays due to neurotoxicity, nephrotoxicity, myelotoxicity, ototoxicity, and others.
- Increase therapeutic index of chemotherapy (dose and/or duration of chemotherapy); this may lead to improved patient benefit.
- No interference with chemotherapy antitumor activity and no protection of the tumor.
- No appreciable additional toxicity; the agent must have a high degree of safety.
- Exploit novel biochemical and pharmacological mechanisms to prevent or mitigate toxicity: thiol/disulfide physiology and pharmacology, and platinum drugs, taxanes, and others.
- Patentability.
- Discovery and development of a novel cost-effective manufacturing process.
- Novel pharmaceutical classification: disulfide chemoprotectant.

Our objective was to attempt to achieve all the foregoing outcomes in this project because if these were all achieved this would represent an ideal drug for chemoprotection. By specifying these drug parameters at the outset, we were able to focus on developing testing methods that would enable us to identify and optimize an ideal drug candidate for development that would fulfill these objectives.

Based on the foregoing, we developed several key testable hypotheses during the drug discovery and development process for the novel chemoprotective agent BNP7787. These hypotheses, as well as several results from both physics-based simulations and laboratory experimentation, are summarized below.

Hypothesis 1 There are at least two chemical species of platinum that differ in chemical reactivity, stability, and other physical and pharmacological properties—one class is responsible for toxicity and the other is responsible for antitumor activity. The differences between these species may be seen by differences in reactivity, drug distribution, and other physical properties. The development of a chemoprotective agent that can distinguish between the two molecular species of platinum may result in prevention or mitigation of platinum toxicities.

At the outset of attempting to discover a safe and effective chemoprotectant, we hypothesized that there are two species of platinum that may exist: (1) molecular species, formed in plasma or cells, which distribute to end organs or tumors that are responsible for antitumor activity; and (2) molecular species that result in end-organ toxicity by their chemical properties including formation, distribution, untoward biological interactions, and metabolism. We sought to discover a compound that would effectively prevent the common and important toxicities arising from platinum chemotherapy without adding intrinsic toxicity or interfering with the antitumor activity of chemotherapy. We formulated this hypothesis to help us to focus on discovering a new drug to prevent platinum toxicity based on these operating principles. By formulating a hypothesis composed of these concepts and definitions, we could design and perform prospective simulations and experiments.

To study the above hypothesis, we developed physics-based computer models of platinum-based drugs that

would have sufficient accuracy and precision in terms of experimental correlation. This initially presented a formidable challenge, which is the relativistic error problem of the platinum atom. The relativistic error problem in ab initio quantum mechanics is based on the Theory of General Relativity, wherein the velocity of the core electrons of the platinum atom approach the speed of light. This problem prohibits the achievement of accuracy and precision of platinum drug models when using conventional quantum mechanical methodology. The conventional methodology is based on the concept that the motion of electrons with respect to atomic nuclei is so much faster that it can be approximated well by keeping the nuclei stationary while allowing the electron distribution to change. To overcome this problem and to test our hypothesis, we had to develop new quantum mechanical methods in order to study the potential toxicity mechanisms of platinum-based drugs. We were successful in developing a new quantum mechanical methodology for platinum-based drugs that produced very accurate and experimentally verifiable simulations [16].

As a result of this research, we were able to accurately simulate the thermodynamic and chemical properties of platinum drugs and their proposed metabolites. We found through chemical transition state simulations that platinum species would form monoaquo and monohydroxy platinum species readily, in agreement with numerous prior experimental studies. In contrast to the literature, we also found that several of the proposed platinum structures were not thermodynamically predicted to form under physiological conditions. In particular, the most unfavored platinum chemical species was diaquo platinum, which we predicted would not form under physiological conditions in plasma [11]. From simulations, we found that monoaquo platinum was far more reactive than monohydroxy platinum with biological targets containing nitrogen or sulfur. We also determined that the water-solubility changes and the chemical stability of platinum species would appreciably increase as a consequence of monoaquation. This observation was predicted to have an impact on the toxicity and distribution of the drug because hydrated platinum is far more polar in this chemical state. We predicted that under physiological conditions the monohydroxy platinum form would have decreased chemical reactivity relative to the monoaquo platinum species and that this species would be the only species forming under physiological conditions in plasma [11].

These simulations were verified by comparison with experimental outcomes from high-field nuclear magnetic resonance, X-ray crystallography, infra-red spectroscopy, and high-performance liquid crystallography (HPLC), as well as in animal toxicology and, ultimately, initial verification in human subjects of platinum pharmacology, which was predicted based on quantum mechanical descriptions of the most probable thermodynamically governed chemical transformations of cisplatin [11].

Hypothesis 2 Monohydrated platinum species are the primary metabolites of platinum drugs and represent the activated platinum species that are primarily responsible for platinum toxicity.

We hypothesized that the monohydrated platinum species that formed in the plasma would be responsible for toxicity with the rank order of toxicity being monoaquo > monohydroxy > > > parent drug species. This was due to: (1) a much greater formation of monohydrated species and no formation of the diaquo or dihydroxy platinum (thermodynamically favored) would occur under physiological conditions, such as in plasma; (2) the monohydrated platinum species were far more reactive than the parent platinum drug species with model biological targets (nitrogen, sulfur, and oxygen); and (3) because of the greatly increased water solubility and polar nature of monohydrated platinum species, it was postulated that these species would distribute rapidly and to a larger degree to the end organs with high blood flow, thereby leading to greater toxicity than the parent drugs' species alone. To test this hypothesis, we chemically synthesized monohydrated cisplatin and cisplatin and administered these to tumor-bearing animals. The result was a 300% increase in the toxicity of monohydrated species relative to the maximum tolerated dose of cisplatin. The acute toxicities were renal, bone marrow, and gastrointestinal tract (all these involve organs with high blood flow) [11].

Hypothesis 3 Sulfur-containing nucleophiles react selectively and thereby inactivate toxic monohydrated platinum species in contrast to the parent platinum compounds.

To prevent toxicity without compromising antitumor activity, it was essential that the chemoprotective agent be highly selective and efficient in inactivating the monohydrated platinum drug in normal healthy tissue, but not in tumor tissue. The key components for specific chemical discrimination for toxicity protection versus tumor protection were found to be the consequence of specific chemical recognition that would discriminate between the monohydrated platinum and the parent platinum drug. Based on the previous studies using physics-based simulation as well as experimental toxicology testing, we believed that it was highly probable that the monohydrated platinum species was responsible for the toxicity in normal tissues by virtue of its reactivity and physicochemical properties (increased water solubility and chemical reactivity). We determined that monohydrated platinum could be readily and selectively inactivated and form a stable complex with sulfur-containing reactants. These chemical reactions, based on physics simulations, were strongly thermodynamically preferred for the monohydrated platinum species relative to the parent platinum drug (e.g. cisplatin, carboplatin, oxaliplatin) [11]. This is a critical observation because it was interpreted that the free thiol would directly and selectively react with the highly water-soluble monohydrated platinum species that are responsible for

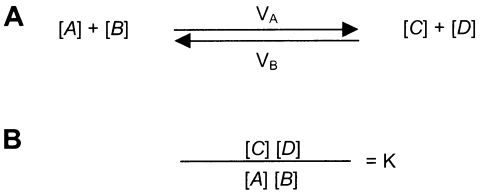

Fig. 1A, B The Law of Mass Action states that in a reversible reaction, the velocity (V_A and V_B) of a reaction is proportional to the product of the molar concentrations of the reactants (**A**) and (**B**). At equilibrium, the concentrations attained when the velocities in the two directions are equal and no further net changes in the concentrations of the reactants occur, then **B** applies. The value of K at equilibrium is always the same, regardless of the proportions or concentrations of the reactants initially present. Adapted from Fig. 10b of Riggs DS (ed) (1970) The mathematical approach to physiological problems. MIT Press, Cambridge, p 245, with permission from Lippincott Williams & Wilkins

toxicity, and not undergo reaction with the parent compound that was responsible for the antitumor activity. The kinetics of this reaction were found experimentally to follow a second-order rate, and therefore we could predict that if excess free thiol was in the plasma it would result in a decrease in the total and free cisplatin as well as the monohydrated platinum. It was also predicted that the disulfide form of the drug would not result in this depletion of the monohydrated platinum or cisplatin; this observation was recently verified in pharmacokinetic studies in patients [26].

Hypothesis 4 Nonenzymatic thiol transfer and disulfide exchange reactions are responsible for maintaining the proportional equilibrium of thiols and disulfides in the plasma and the cell (e.g. predominantly oxidized species in the plasma and reduced species in the cell) and this chemical equilibrium is a predictable process for thiol- and disulfide-containing drugs in the absence of enzymatic activity (e.g. thiol transferase, glutathione reductase).

Our hypothesis for nonenzymatic thiol transfer was based on our inability to reproduce published experiments, wherein it was stated that glutathione reductase and thiol transferase mediated the generation of free 2-mercapto ethane sulfonate (mesna) from its disulfide form. We found that neither enzyme was capable of producing mesna directly from its disulfide form. This made us consider new possibilities to explain the reduction and oxidation of sulfur-containing amino acids, glutathione, and drugs in a manner that are consistent with the Law of Mass Action (Fig. 1).

Ab initio quantum mechanical calculations of the chemical transition state of a thiol reacting with a disulfide revealed that an S_N2 (second-order nucleophilic; direct displacement) reaction was predicted to readily occur between a thiol-containing molecule and a disulfide-containing molecule. The predicted products would include a mixed disulfide plus a newly generated free thiol. We postulated several key reactions (Table 1) that were important for nonenzymatic thiol transfer involving oxidized and reduced species of glutathione, cysteine, homocysteine, and BNP7787 or mesna.

Table 1 Predicted key (and residual) reactions for BNP7787 and mesna: nonenzymatic thiol transfer (*MssM* BNP7787; *Rsh* cysteine, glutathione, or homocysteine; *Msh* mesna, *MssR* mesna-disulfide heteroconjugate; *RssR* cystine, oxidized glutathione, homocystine, or mixed disulfides)

Reactants	Products
MssM + Rsh	Msh + MssR + RssR (+ MssM + Rsh)
Msh + RssR	Rsh + MssR + MssM (+ Msh + RssR)
MssM + RssR	No reaction
Msh + Rsh	MssR + MssM + RssR (+ Rsh + Msh)
Msh + Msh	MssM (+ Msh)
Rsh + Rsh	RssR (+ Rsh)

The foregoing reactions have been experimentally confirmed in our laboratory. Figures 2 and 3 present data for the reactions of mesna with cystine and BNP7787 with cysteine, respectively; the HPLC tracings demonstrate the time-dependent product formation from these reactions. These thiol disulfide exchange reactions are integral to understanding normal plasma and cellular physiology and the pharmacological effects that therapeutic thiol and disulfides can exert through these reactions. The rate constants for these reactions will be published elsewhere.

Relevant to the foregoing, it was notable that there are no reductases, thiol oxidases, or thiol transferase enzymes in the plasma. Normal human blood has a high pO_2 due to oxyhemoglobin, which in turn affects the oxidation of a large proportion of the thiols into disulfides. The cell contains thiol transferases and reductases that affect the equilibrium of the above reactions, and the cell maintains a reduced environment via NADPH and NADH reactions. We have also found that there are thiol- and disulfide-containing biological intermediates that are affected and react by these same mechanisms; these interactions are also critical and are exploited in a novel manner by BNP7787.

Hypotheses 5 and 6 The intrinsic clinical toxicity of sulfur-containing chemoprotective agents is mediated by their ability to disrupt the normal plasma and cellular thiol and disulfide equilibrium operating in accordance with the Law of Mass Action. The ability to prevent toxicity from chemotherapeutic drugs such as platinum agents or taxanes, as well as to avoid intrinsic toxicity from the chemotherapeutic agent itself, can be better achieved by the administration of a disulfide-containing chemoprotective agent.

In parallel to the foregoing, we formulated two hypotheses regarding physiological and pharmacological conditions that would have to be maintained for a nontoxic and efficacious chemoprotective agent that would utilize nonenzymatic thiol transfer reactions. It is notable that more than 60 different compounds have been studied for the purpose of chemoprotection against platinum

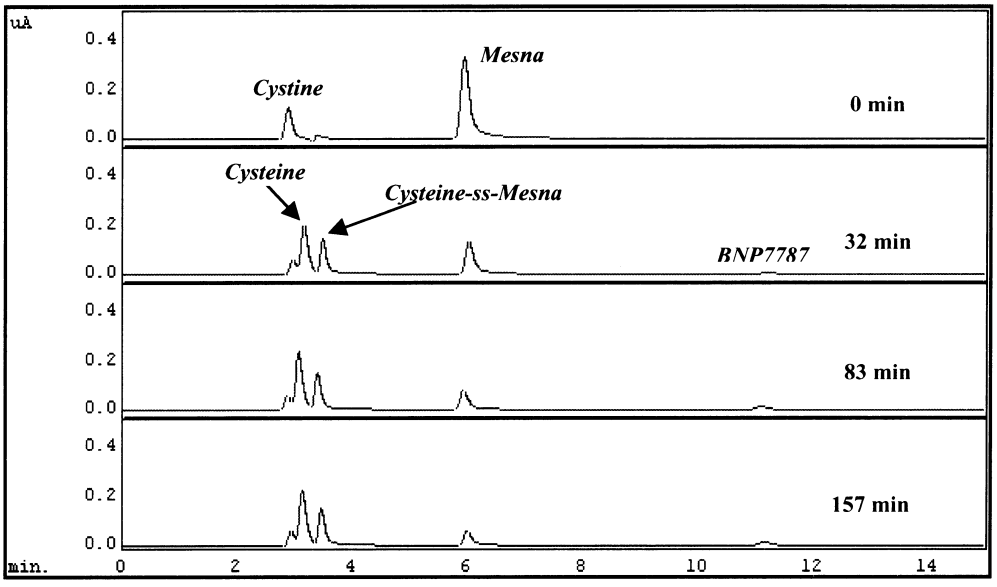

Fig. 2 HPLC electrochemical detection (dual electrode) of reaction between 100 μ*M* cystine and 100 μ*M* mesna in 100 m*M* phosphate-buffered saline (pH 7.4) at 37°C

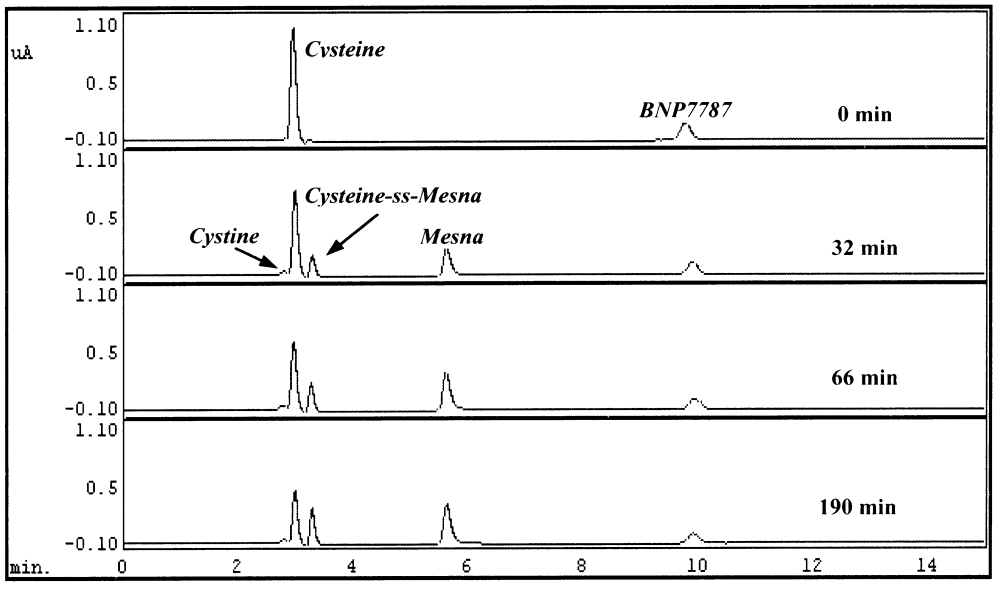

Fig. 3 HPLC electrochemical detection (dual electrode) of reaction between 100 μ*M* cysteine and 100 μ*M* BNP7787 in 100 m*M* phosphate-buffered saline (pH 7.4) at 37°C

toxicity, and that only four of these compounds entered clinical testing. Of these agents, only one was approved for use in humans [11]. This hypothesis was based on the observations of toxicity and tumor protection noted with earlier chemoprotective agents, namely hypotension, nausea, vomiting, tachycardia, diaphoresis, diarrhea, and other toxicities associated with the administration of the platinum protective agents sodium thiosulfate, diethyl dithio carbamate, and WR2721 (amifostine). This hypothesis was formulated in part as a result of writing a prescription for captopril for a patient. In the process of reviewing current information on this drug (this patient was receiving several different medications and there was concern about potential drug–drug interactions), it was noted that captopril contains a free thiol and this drug can produce some of the same adverse effects as sulfur chemoprotectants and is also metabolized to disulfide and cysteine disulfide forms. This observation led to the concept that the prevention or mitigation of intrinsic toxicity associated with previous chemoprotective agents could be achieved by administration of the disulfide form to patients, because such an approach would not be disruptive to the normal plasma thiol and disulfide physiology. In addition, it was reasoned that the chemical reactivity of a disulfide in the plasma would be far less than a free thiol-containing drug, thereby reducing the probability of an untoward drug interaction with chemotherapy and tumor protection.

This hypothesis was formulated based in part on nonenzymatic thiol transfer reactions and the integrated application of the Law of Mass Action for normal plasma and cellular thiol and disulfide physiology, which describes the critical relationships that must be maintained between the plasma and cellular disulfide and

thiol proportions; this important physiological and pharmacological relationship has not been previously described. This hypothesis and the proportional relationship of normal plasma and cellular thiols and disulfides are illustrated in Figs 1 and 4 and Table 2.

Using the formula, the estimated equilibrium value for K_{plasma} is about 19 and for $K_{intracellular}$ is about 0.01 for the normal ratio of disulfides to free thiols in the plasma and cell, respectively. Accordingly, in relative terms, the consequences of administration of a free thiol versus its corresponding disulfide can be predicted: the administration of a free thiol-containing drug will result in a greater proportional change in K_{plasma} (decrease), whereas there will not be such a proportional change in K_{plasma} ($<5\%$) with the administration of the corresponding disulfide. This formula illustrates that even with the administration of an enormous amount of disulfide, there cannot be an enormous change in K_{plasma}; the maximum proportional change would only be 5%. This formula also predicts, assuming 100% transport into cells (unlikely for most drugs), that the interrelationship of thiol and disulfide proportions comprised in K_{plasma} in the plasma will predictably affect $K_{intracellular}$ in the cell in a predictably proportional manner. Accordingly, the administration of a free thiol-containing compound will lead to a temporal decrease in K_{plasma} and will thereby result in a predictably large increase in $K_{intracellular}$. This relationship predicts that both K_{plasma} and $K_{intracellular}$ will be more greatly perturbed by a given dose of a free thiol-containing drug than when the same or even a greater amount of a corresponding disulfide is administered. It is also clear that even when a large amount of disulfide is administered, the maximum change in the plasma disulfide proportion can only be 5%, whereas with a thiol-containing drug, the maximum change in the thiol proportion can be as much as 95%. This relationship has important new implications for the accurate description of physiological and pharmacological relationships of thiols and disulfides in the plasma and cells and the effects of thiol- or disulfide-containing drugs in these compartments.

$$\frac{\dfrac{RssR_{[plasma]}\ (95\%\ oxidized)}{Rsh_{[plasma]}\ (5\%\ reduced)}}{\dfrac{RssR_{[intracellular]}\ (1\%\ oxidized)}{Rsh_{[intracellular]}\ (99\%\ reduced)}} = \frac{K_{[plasma]}}{K_{[intracellular]}}$$

Fig. 4 Relationship of the ratio of physiological disulfides and thiols in the plasma and cell. Disulfides predominate (about 95%) in the plasma, an oxidized environment, while free thiols predominate (about 99%) in the cell, a reduced environment

This formula is a descriptive hypothesis that free thiol-containing drug administration leads to an increase in plasma free thiol which concurrently results in a decrease in plasma disulfide concentration. This delineates the differences in the disruption of the physiological balance of plasma and cellular thiols and disulfides in accordance with the Law of Mass Action. An integral component of this hypothesis is that any proportional increase in the plasma thiols and disulfides thereby proportionally decreases the concentration of intracellular free thiols, a phenomenon that is accompanied by a

Table 2 The Law of Mass Action relationship for plasma and cellular disulfide and thiol proportions, and concentrations and resulting outcomes following administration of a disulfide-containing drug versus a thiol-containing drug (*RssR* cystine, oxidized glutathione, homocystine, BNP7787 or mixed disulfides; *Rsh* cysteine, glutathione, mesna or homocysteine) $\frac{[RssR/Rsh]_{plasma}}{[RssR/Rsh]_{intracellular}} = \frac{95\%-ss-/5\%-sh}{1\%-ss-/99\%-sh} = \frac{K_{plasma}}{K_{intracellular}}$

Administration of disulfide [RssR]-containing drug	Administration of thiol [Rsh]-containing drug
Not physiologically disruptive	Physiologically disruptive
Avoids or lessens perturbation of plasma disulfide and cellular free thiol equilibrium	Lowers plasma disulfide fraction and significantly perturbs equilibrium between plasma and tissues
Nonenzymatic thiol transfer predominates intracellularly: reduced toxicity, less risk of plasma drug–drug interaction	Greater potential for toxicity and drug–drug interactions
Disulfide favored by high pO_2, lack of plasma reductases, H_2O solubility	Key chemical reactions involve thiol drug and cystine, homocystine and oxidized glutathione
	Mediated by nonenzymatic thiol transfer (S_N2 reaction) in the plasma
Increases disulfide concentrations in plasma and total cellular sulfur (protective)	Increases plasma thiol proportions and decreases intracellular free proportions with reciprocal increase in intracellular disulfide proportions (toxic)
Less perturbation in disulfide–thiol equilibrium ratio	Greater perturbation of normal physiological balance of disulfide–thiol equilibrium ratio
Nontoxic, less risk of drug interaction	Greater risk of drug interaction by free thiol
Addition of disulfide drug increases total disulfide concentration	Addition of thiol drug (greatly) increases total thiol concentration
Plasma disulfide and intracellular thiol concentrations increase, but the maximum percentage change in plasma disulfide proportion is only 5% (nontoxic); changes much less than when the same dose of the free thiol form of the same drug is administered	Plasma free thiol and intracellular disulfide concentrations increase, but the maximum percentage change in plasma thiol proportion is as much as 95%; changes much greater than when the same dose of the disulfide form of the same drug is administered (toxic)

reciprocal proportional increase in the intracellular disulfide concentration. This outcome may explain, in part, the observed toxicities associated with the administration of drugs that possess free thiols or those that are rapidly metabolized to free thiols in plasma. This hypothesis also suggests that the administration of a disulfide-containing drug greatly increases the disulfide concentration in plasma but cannot greatly perturb the percentage of disulfide and free thiol (in the plasma) or in the cell as compared with an identical dose of the free thiol form of the same drug. The outcome is a relatively nontoxic increased free thiol concentration in the cell with a concomitant proportional decrease in cellular disulfide.

The experimental evidence including safety data from multiple species (rats, dogs, and humans) on the relative toxicity of mesna (a free thiol-containing drug) and its disulfide form (BNP7787), and pharmacokinetic data on BNP7787 and mesna in humans and animal models corroborate this relationship of normal physiological concentrations reported for plasma thiols and disulfides and the relative toxicities of these two compounds. In normal plasma, the clinically relevant thiols including cysteine (200–300 μM), glutathione (2–5 μM), homocysteine (5–15 μM), and cysteinyl glycine (100 μM) are present primarily (>93%) in oxidized forms [15]. In contrast, the cell interior is a highly reduced environment in which thiols including cysteine, cysteinyl glycine, homocysteine, and glutamyl cysteine predominate as reduced species, and the intracellular concentration of glutathione is 1–11 mM [8, 9, 21, 24] and is reported to exist 99% as free thiol [5]. The relative dose-related toxicities of intravenously administered mesna, BNP7787, and WR2721—a phosphorothiol that is rapidly metabolized ($t_{1/2} = 1$ min) in plasma to a free thiol—in animals and humans are shown in Table 3 [11].

Hypothesis 7 Tubulin is a critical biological target of monohydrated platinum and taxane-type drugs that may be responsible for drug-induced toxicity; such toxicity is manifested by cellular necrosis.

Tubulin is a ubiquitous cellular protein that is responsible for many essential cellular functions and is a highly abundant cellular protein and can comprise 10% or more of total cellular protein content [1]. It was recognized in the early 1980s that taxanes induce abnormal tubulin polymerization and this was postulated to be responsible for their antitumor activity [22, 23]. Because this interaction was well known, and the clinical features of neurotoxicity for platinum and taxanes have similar features, we investigated the structural properties of human tubulin subtypes in an effort to discern whether there might be some common mechanisms for these two different drugs. We constructed three-dimensional atomic structures of human tubulin subtypes based on X-ray structures of animal tubulin by homology modeling, protein folding, and molecular dynamics. We noted that there were several surface-accessible cysteine/cystine groups on both the α and β subunits; many of these had the same accessibility on the various tubulin subtypes. We also observed that tubulin contains several cysteines that are surface-accessible and this led us to consider the possibility that platinum species could modify tubulin and interfere with its polymerization. In looking carefully at these structures, we also considered that the free thiol metabolite of BNP7787, mesna, could intercept these toxic species of platinum prior to this detrimental interaction. This led to experiments that tested these concepts with the result that mesna is protective against the tubulin toxicity mediated by monohydrated platinum species [12, 13]. We also found that BNP7787 normalized the tubulin hyperpolymerization induced by paclitaxel and the effects of BNP7787 in preserving normal tubulin morphology in the presence of paclitaxel were indistinguishable from normal tubulin morphology by electron microscopy [12]. There is no apparent binding site on the surface of tubulin for BNP7787; the effect appears to be a consequence of

Table 3 Summary of acute toxicity studies following intravenous administration of BNP7787 or mesna (free thiol) in animals and humans

Drug	Group	Dose	Comments	Reference
Mesna	Rats	$LD_{50} = 1800$ mg/kg	–	2
		$LD_{25} = 1500$ mg/kg	–	11
BNP7787	Rats	4000 mg/kg (nonlethal)	Dose safety ratio BNP7787:mesna >2.7:1	11
Mesna	Dogs	400 mg/kg (100% lethal)	–	2
BNP7787	Dogs	4000 mg/kg (nonlethal)	Dose safety ratio BNP7787:mesna ~10:1	–[a]
Mesna	Humans	2.4 g/m^2 (toxic)	Diarrhea (83%), headache (50%), pain (50%), nausea (33%), decreased blood pressure (17%)	Physicians' Desk Reference, American Hospital Formulary Service
BNP7787	Humans	41 g/m^2 (no dose-limiting toxicity observed in three phase I trials)	Dose safety ratio BNP7787:mesna >15:1	–[b, c, d]

[a]BioNumerik #SBL-29–31, a Good Laboratory Practice study conducted at Shin Nippon BioMedical Laboratories, Ltd., Kagoshima, Japan
[b]BioNumerik Good Clinical Practice phase I study conducted in the USA (Roswell Park Cancer Institute and University of Chicago Cancer Center)
[c]BioNumerik Good Clinical Practice phase I study conducted in Europe
[d]BioNumerik Good Clinical Practice phase I study conducted in Japan

concentration-dependent surface-charge neutralization of tubulin in the presence of paclitaxel as well as the maintenance of normal oxidation states of tubulin cysteines.

Because tubulin plays an integral role in cells, including cancer cells, the differential effects of BNP7787 in protecting and modulating tubulin are due to transport and uptake of the drug, which we have found to be the highest in the kidney, marrow, intestine, salivary glands, and bladder following administration of radiolabeled compound. We have also found that BNP7787 does not interfere with paclitaxel-induced apoptosis [13] nor cytotoxicity [3, 12] of a variety of anticancer drugs—platinum drugs, taxanes, vinca alkaloids, epothilones and others. The mechanisms for the apparently selective cytoprotection by BNP7787 involves its distribution into these organs and the relative lack of distribution to organs with lower blood flow combined with the fact that the drug is highly negatively charged and cannot cross cell membranes readily. Other mechanisms involve the drug's ability to protect against drug-induced necrosis and interference with normal tubulin function, while not interfering with apoptosis in tumor cells.

Clinical development and a preliminary summary of phase I studies with BNP7787

In phase I trials of BNP7787 with paclitaxel (175 mg/m^2) and cisplatin (75 mg/m^2) or single-agent cisplatin (75 mg/m^2) administered every 21 days, no grade 3 or 4 neurotoxicity has been observed in more than 100 patients. For patients treated with the combination of paclitaxel and cisplatin, the incidence of grade 2 neurotoxicity was 16% for all BNP7787 dose levels (4.1–41.0 g/m^2) and 12% at a dose of 18.4 g/m^2. The incidence and severity of neurotoxicity appears markedly reduced with BNP7787 in combination with chemotherapy compared with the neurotoxicity using a similar chemotherapy regimen (no BNP7787) reported in the literature as 42% for grade 2 and above and 21% for grades 3 and 4 [4]. BNP7787 doses of up to 41.0 g/m^2 have been administered intravenously without dose-limiting toxicity in humans. BNP7787 appears to be a relatively nontoxic drug based on clinical observations to date.

The objective tumor response rate observed in a phase I study in patients with advanced non-small-cell lung cancer was approximately 45% (9 of 20 patients with a partial response) using a maximum of three cycles of paclitaxel (175 mg/m^2) and cisplatin (75 mg/m^2). In another phase I study, it is notable that 2 patients (one with central nervous system metastasis of an adenocarcinoma most probably due to lung cancer primary and one with adenocarcinoma of unknown primary) experienced a complete response and remained in complete remission for 18 and 8 months, respectively. Currently, 5 patients have completed at least three cycles of treatment with paclitaxel and cisplatin without the administration of normal saline prophylaxis for cisplatin-induced nephrotoxicity; none of these patients has experienced dose-limiting nephrotoxicity, and notably no severe neurotoxicity. The results from these studies will be reported in detail elsewhere.

Based on clinical and preclinical studies, there is a reasonable basis to believe that BNP7787 administration may substantially prevent and lessen the severity of paclitaxel and cisplatin neurotoxicity, and cisplatin-induced nephrotoxicity and ototoxicity as well as other chemotherapy-associated toxicities, and might thereby allow increases in the number of doses and total dose of paclitaxel and cisplatin. Completion of the ongoing phase I study using no normal saline prophylaxis for platinum-induced nephrotoxicity will help define an important use of BNP7787: to obviate the need for saline hydration and thereby allow the safe, more-convenient, and less-costly administration of saline to patients who receive cisplatin. BNP7787 is currently in phase III trials in the USA (where the Food and Drug Administration has granted Fast Track Designation for the prevention of paclitaxel-associated neurotoxicity) and in Europe for the prevention of paclitaxel- and cisplatin-induced neurotoxicity. Phase III studies of BNP7787 will commence in Japan in 2003. Additional future trials are being planned with other chemotherapy drugs and combinations in other patient populations.

Summary and conclusions

Physics-based molecular simulations will play an increasingly important role in improving the discovery and development of new drugs for cancer treatment. Drug discovery is a complex learning and creative endeavor that can be made more effective by multidisciplinary research operations and the application of technologies that can help effectively to reduce the time and cost of failures in the trial and error process of discovery. The evidence to support the concept that drug discovery is based on luck or probability game theory appears to be very limited, based on the past 20 years of research, and the current productivity and operational costs for the pharmaceutical industry. There is increasing evidence that physics-based molecular simulations have played an important role in the discovery and development of new drugs. The primary limitations of this approach have been in software and computing power, but these appear to be less problematic today and will be less so in the near future. It is important to integrate this technology into pharmaceutical discovery and development operations so that new hypotheses for pharmaceutical intervention can be generated and tested concurrently by simulation and experiment—this will be true for new compounds discovered by synthesis or from natural products. Future advances in all these areas will have a substantial impact on the effectiveness of discovery and development of safe and effective new drugs by medical and pharmaceutical researchers.

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