



HAL
open science

Ready for a comeback of natural products in oncology

Christian Bailly

► **To cite this version:**

Christian Bailly. Ready for a comeback of natural products in oncology. *Biochemical Pharmacology*, 2009, 77 (9), pp.1447. <10.1016/j.bcp.2008.12.013>. <hal-00493481>

HAL Id: hal-00493481

<https://hal.science/hal-00493481v1>

Submitted on 19 Jun 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Accepted Manuscript

Title: Ready for a comeback of natural products in oncology

Author: Christian Bailly

PII: S0006-2952(08)00890-3
DOI: doi:10.1016/j.bcp.2008.12.013
Reference: BCP 10035

To appear in: *BCP*

Received date: 18-9-2008
Revised date: 30-11-2008
Accepted date: 9-12-2008



Please cite this article as: Bailly C, Ready for a comeback of natural products in oncology, *Biochemical Pharmacology* (2008), doi:10.1016/j.bcp.2008.12.013

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

“COMMENTARY” *Biochemical Pharmacology* (revised version 30 November 2008)

Ready for a comeback of natural products in oncology

Christian Bailly

Institut de Recherche Pierre Fabre, Parc Technologique du Canal, 3 rue des Satellites, B.P.
94244, 31432 Toulouse Cedex 4, France.

Corresponding Author : Christian Bailly, Ph.D., Pierre Fabre Research Institute, Parc
Technologique du Canal, 3 rue des Satellites, B.P. 94244, 31432 Toulouse, Cedex 04, France.
Phone: (33)534 32 14 44; FAX: (33)534 32 14 34; E-mail: christian.bailly@pierre-fabre.com

Running title : Comeback of natural products in oncology

Key Words : Cancer, natural products, targets

Abbreviation List : CRC, colorectal cancer; FDA, Food & Drug Administration; high
throughput screening, HTS; NP, natural products; NSCLC, non small cell lung cancer; SAR,
structure-activity relationship.

ABSTRACT (186 words)

Since the late 1990s and the rapid expansion of monoclonal antibodies and synthetic protein kinase inhibitors in oncology, anticancer natural products fell out of fashion with the pharmaceutical industry. But in 2007 with the approval of three new drugs derived from natural products, the emergence of promising antitumor compounds from microorganisms (e.g. alvespimycin, salinosporamide) and the growing importance of new formulations of known natural product-derived drugs (nanoparticle formulations, oral forms), we are witnessing a new wave for natural products in oncology. The recent approval of the microtubule-targeted epothilone derivative ixabepilone (Ixempra[®]), the DNA-alkylating marine alkaloid trabectedin (Yondelis[®]) and the inhibitor of mTOR protein kinase temsirolimus (Torisel[®]) is emblematic of the evolution of the field which combines the long established finding of conventional cytotoxic agents and the emergence of molecularly targeted therapeutics. These three examples also illustrate the increasing importance of microbial sources for the discovery of medically useful natural products. The contribution of innovative biological targets is also highlighted here, with references to proteasome inhibitors and novel approaches such as manipulation of mRNA splicing. Altogether, these observations plead for the return of natural products in oncology.

1. Introduction

The space race and the war on cancer both begun in the 1950s. When Yuri Gagarin first went into orbit around the earth in 1961, key antitumor antibiotics like doxorubicin and mitomycin were discovered. When Neil Armstrong put his foot on the moon on July 20 1969, some novel antitumor agents such as bleomycin and vincristine were just launched. Over the past 50 years, natural products (NP, here the term is restricted to small molecules) have been the cornerstone of anticancer pharmacology. The discovery of these antitumor NP opened the route to the “forty glory cytotoxic”, 4 decades of cytotoxic agents, among which a significant number of molecules from plants and microorganisms still used today for the treatment of cancer (Fig. 1). Many of these old drugs remain largely prescribed and have saved or prolonged the life of millions of patients with cancer. But at the end of the 1990s, a novel onco-pharmacological universe was discovered, with the use of targeted therapeutics made of receptor-specific small (imatinib) or large (rituximab) molecules. Immediately, the antitumor pharmacology community embraced the new concept and this vision of molecular target-based drug discovery (or reverse pharmacology) became a standard. At this stage, the search for natural products in oncology declined and there was hope to envisage rapidly a personalised treatment of cancer with rationally designed magic bullets, silico- or omico-controlled. The objective remains today but the route is long and progresses are slow. There is no doubt that the treatment of cancers has profoundly changed, with the advances of targeted therapies. But major differences remain between the different types of tumors. Patients suffering from chronic myeloid leukaemia have a good probability of long-term survival, thanks to the discovery of specific tyrosine kinase inhibitors like imatinib (Gleevec[®]), dasatinib (Sprycel[®]) or nilotinib (Tasigna[®]). In sharp contrast, the therapeutic options for patients with advanced melanoma remain quite limited and the survival prognostic is still extremely low. The activity of dacarbazine (DTIC) against metastatic melanoma was first noticed in the early 1970s [1] and 36 years later, this product remains a standard: where is the progress? For the majority of solid tumors, in particular for advanced and metastatic grades, treatment modalities remain often palliative and very insufficient, despite the development of newer drugs supposed to provide novel therapeutic options. These drugs are effective, otherwise they would not be on the market, but in many cases, they only prolong the life of patients for several weeks or a few months, rarely for years. There is a strong need for novel anticancer drugs effective against solid tumors, especially at advanced stages of the disease. The need is large and urgent for nearly all solid tumors. Just one example: every 3.5 minutes,

someone is diagnosed with colorectal cancer (CRC); every 9 minutes, someone dies from CRC; and every 5 seconds, someone who should be screened for CRC is not [2]. We definitely need new drugs, as well as new combinations, new strategies to select patients and to evaluate treatment responses. As regards drugs, I believe that NP should continue to be considered as one of the most important sources of innovative products. Astronauts apparently plan to go back to the moon in the near future, possibly 2011, to learn new things, develop innovative research programs and demonstrate that they can essentially "live off the land". By analogy, a new vision for NP space exploration can be envisioned to drive drug discovery and spur new medical advancements, especially in the field of cancer through the exploration of novel biodiversity sources and emerging targets. In this commentary, I tried to analyze the current situation with the place and role of NP in modern oncology, discuss about recent evolutions and some perspectives. This is not an exhaustive survey of NP in cancer, there are excellent recent reviews for that [3,4] but essentially a personal vision of the field at the interface between chemistry and biology.

2. An excellent track record for natural products in oncology

Old good drugs from plants and microbes remain essential. Since the 1950s, a large number of novel antitumor drugs have been identified and validated clinically (Fig. 1). However, the proportion of NP is certainly decreasing, mainly due to the advance of small synthetic molecules (in particular kinase inhibitors) and the fast growing development of monoclonal antibodies. Nevertheless, NP remain essential and novel molecules of marine or terrestrial origins are regularly approved for the treatment of cancers. Examples of recently launched NP and derivatives will be discussed below. Known molecules too continue to contribute considerably to cancer chemotherapy. The anthracyclines such as doxorubicin, epirubicin and daunorubicin cannot be ignored. This is especially the case for doxorubicin (Adriamycin[®]) extensively used for the treatment of breast cancer, despite the risk of cardiotoxicity particularly in patients with underlying cardiac issues or the elderly. This drug is also frequently associated with monoclonal antibodies, with trastuzumab for breast cancers, with rituximab for lymphoma, for examples. There are numerous chemotherapy regimens associating doxorubicin with chemo- and bio-therapeutics. In the early seventies there was initial clinical reports on the use of doxorubicin in the treatment of Hodgkin disease, leukaemia, breast, lung and cervical cancers. In one way, it is regrettable to admit that a quarter of century later, this bacterial product qualified in 1974 as a new drug with significant

clinical activity [5], remains one of the most important molecule of the anticancer armamentarium. The situation is comparable with mitomycin C, discovered in the mid-1950s [6], used clinically in the early 1960s and still prescribed nowadays for lung, gastric colorectal and pancreatic cancers for examples. This antibiotic, originally isolated from *Streptomyces caespitosus*, is a bioreductive drug that requires metabolic activation by cellular reductases, such as NAD(P)H:Quinone oxidoreductase-1 (NQO1), for activity. It is widely used systemically for the treatment of malignancies, and has gained popularity as topical adjunctive therapy in ocular and adnexal surgery over the past two decades. In the same vein, one can refer to bleomycin and actinomycin, two other old antitumor antibiotics still on the shelves of clinical oncologists. A parallel situation can be established with drugs derived from plants. Vinorelbine (Navelbine[®]), arguably the major semi-synthetic tubulin-binding vinca alkaloid, first approved in Europe in 1989 and in the USA in 1994 for the treatment of non-small cell lung cancer (NSCLC) then of advanced breast cancer, remains today extensively used worldwide. Similarly, paclitaxel (Taxol[®]) and its rival docetaxel (Taxotere[®]) continue to dominate the cytotoxic market.

3. New formulations of known natural products

New forms of known natural products are being continuously developed to facilitate the handling of the drugs (for example for poorly soluble compounds) or limit their side toxicities. This is typically the case for the two taxanes paclitaxel and docetaxel, both effective but which often encounter undesirable side effects as well as drug resistance. Second generation taxanes are being developed such as ortataxel or more recently TPI 287 (Tapestry Pharmaceuticals) engineered to overcome multi-drug resistance and to bind to mutant tubulin. Taxane-containing polymers are also investigated such as paclitaxel poliglumex (Opaxio[®] formerly known as Xotax[®]) or Taxoprexin[®] made by linking paclitaxel to the natural fatty acid DHA (docosahexaenoic acid). The leading compound has been ABI-007 (Abraxane[®] from Abraxis Bioscience), approved by the U.S. Food & Drug Administration (FDA) in January 2007 for the treatment of metastatic breast cancer and which is an albumin-stabilized, nanoparticle formulation of paclitaxel for injectable suspension. This form was found to have significantly less toxicity than Cremophor-containing paclitaxel in mice [7,8]. Solvent-based formulations of taxanes are commonly associated with hypersensitivity reactions, neutropenia, and neuropathy. Patients with metastatic breast cancer treated with this nanoparticle formulation (mean particle size of about 130 nm) achieved a significantly higher objective

response rate and time to progression than those treated with Cremophor-containing paclitaxel. This nab-paclitaxel form may therefore enable clinicians to administer paclitaxel at higher doses with less toxicity than is seen with Cremophor-containing paclitaxel [9]. Similar albumin-based drug development are ongoing with other water-insoluble anticancer agents such as docetaxel and rapamycin [10,11]. A similar trend can be evoked with the camptothecin-derived topoisomerase I inhibitor topotecan (Hycamtin[®]) initially approved in 1996 for the treatment of small cell lung cancer (SCLC) after failure of first-line chemotherapy and metastatic ovarian carcinoma after failure of initial or subsequent chemotherapy. Since the approval of injectable topotecan for the second-line treatment of SCLC, new formulations have been developed. In October 2007, more than 10 years later, a new oral formulation of the drug as capsules for the treatment of patients with relapsed small cell lung cancer was proposed [12]. Oral topotecan will represent a good candidate for combination therapy with other i.v. or oral chemotherapy agents, monoclonal antibodies, and small molecule tyrosine kinase inhibitors. In the same vein, oral availability and administration of some of the newer CPT analogues, including diflomotecan (BN80915) and gimatecan (ST1481), have also shown promising results.

One of the most typical example of reformulation is that of pegylated liposomal doxorubicin (Doxil[®] from Tibotec Therapeutics; Caelyx[®] from Schering-Plough) which has demonstrated efficacy in the treatment of recurrent ovarian cancer. The liposome-encapsulated form improves doxorubicin penetration into tumors and decreases drug clearance, thereby increasing the duration of the therapeutic effect. Pharmacokinetic studies have indeed revealed that the liposomal form results in a longer half-life with less free drug available for tissue distribution than conventional doxorubicin. This liposomal formulation of doxorubicin reduces toxicity, specifically the cardiac effects commonly seen with anthracycline antitumor drugs but tolerability is often non uniform among patients. Very recently, a liposome-encapsulated cytarabine/daunorubicin combinations with a molar ratio of 5:1, designated CPX-351 (from Celator Pharmaceuticals), has been granted orphan drug designation by the FDA for the treatment of acute myeloid leukemia. Liposomal formulations of platinum drugs are also studied.

Another practical approach to improve the efficacy or facilitate the handling and tolerability (and patients' preferences) of a natural product consists to develop an oral regimen. Most cytotoxics are administrated intravenously, but some are also available in an oral formulation. This is the case for oral vinorelbine formulated in a soft gelatin capsule, with a predictable

bioavailability of about 40% [13] and comparable efficacy to the i.v. form with respect to response rate, progression-free survival and overall survival [14]. This orally active vinca alkaloid (Fig. 2) offers novel opportunities for cancer treatment, both in NSCLC and in metastatic breast cancer. Oral formulations can reduce the incidence of side effects associated with an i.v. line (extravasation, pain, local infection, thrombosis), contribute to resource sparing for the health care system and for the patient [15] and to alleviating the burden on his/her relatives. Even when combined to intravenous treatments, the oral formulation of vinorelbine is preferred to its intravenous counterpart by 74% of patients [16]. By reducing constraints related to treatment administration, oral agents improve the global efficiency of cancer treatment. Their use is increasing in oncology [17].

4. Novel approved NP drugs and promising clinical candidates

As far as I know, no novel category of NP was approved by the FDA for the treatment of cancer during years 1997-2006 (Fig. 3). In the same time, resources attributed to NP research in oncology by most major pharmaceutical companies were reduced. During that period, efforts were essentially focused on biotherapies and targeted synthetic small molecules. However, the field of NP remained active with the clinical development of a few innovative molecules, among which 3 key products, ixabepilone, temsirolimus and trabectedin now registered in the USA and/or in the EU. They are briefly mentioned here:

- **Ixabepilone** (BMS-247550, Ixempra[®] from Bristol-Myers-Squibb). Drugs that target microtubules, including vinca alkaloids (vinorelbine, vincristine) and taxanes (paclitaxel and docetaxel), are arguably the most commonly prescribed anticancer therapies. The use of these “old good drugs” may however be limited by side toxicities and/or resistance induced by efflux proteins. Novel antimicrotubule agents, better tolerated and active in the setting of drug resistance have been searched. This is the context which lead to the discovery of the macrolide antibiotics epothilones, produced by the myxobacterium *Sorangium cellulosum*, as a novel class of microtubule-stabilizing agents discovered in 1995 [18]. Epothilones A-B exhibit potent *in vitro* anticancer activity, against taxane-resistant cell lines, but their *in vivo* activity is modest, owing to a poor metabolic stability and unfavorable pharmacokinetics. The synthesis and testing of more than 300 semi-synthetic epothilone analogues led to the rational design of ixabepilone, which displays reduced susceptibility to a range of common tumor resistance mechanisms. This novel antimicrotubule agent acts in a similar manner to taxanes,

stabilizing microtubules and resulting in arrested tumor cell division and apoptosis. But, in contrast to taxanes, ixabepilone has reduced susceptibility to resistance due to P-gp overexpression, tubulin mutations, and alterations in β -tubulin isotype expression. It demonstrated in vivo antitumor activity in a range of human tumor models, including tumors resistant to anthracyclines and taxanes [19-21]. In October 2007, ixabepilone administered by intravenous infusion, received FDA approval for the treatment of metastatic or locally advanced breast cancer. That is 12 years from the discovery of epothilone B to the launch of ixabepilone for the treatment of cancer. Other epothilone derivatives, such as sagopilone (ZK-EPO) and patupilone (epothilone B) are being developed [22].

- **Trabectedin** (Ecteinascidin-743, Yondelis[®] developed by PharmaMar in partnership with Johnson & Johnson). ET-743 is a tetrahydroisoquinoline alkaloid derived from the Caribbean marine tunicate *Ecteinascidia turbinata* (sea-squirt) but now produced semi-synthetically starting from cyanosafraicin B extracted after fermentation of the marine bacterium *Pseudomonas fluorescens*. It functions as a DNA minor groove alkylating agent specific of guanine residues at the N2 position, producing specific adducts that represent a unique challenge to the DNA repair machinery [23]. Alkylation results in DNA bending which affects various transcription factors involved in cell proliferation, particularly via the transcription-coupled nucleotide excision repair system. Trabectedin has been recently approved in the EU as a second-line treatment for advanced soft tissue sarcoma in patients who have failed on anthracyclines and ifosfamide, or who are unsuited to receive these agents. It also has orphan drug status in soft tissue sarcoma in the US and in ovarian cancer in the US and EU, and is under investigation as combination therapy in patients with recurrent ovarian cancer [24]. Hopefully, trabectedin has opened up the way to the successful development of other marine NP. There are several marine drug candidates (derived from dolastatin 10, bryostatin 1, didemnin B, kahalalide F, halichondrin B, etc) currently in clinical trials [25,26]. The development of marine toxins remains a difficult challenge but hopefully the next decade will offer a prominent place to sea products [27].

- **Temsirolimus** (CCI-779, Torisel[®] from Wyeth) is the first inhibitor of the protein kinase mTOR (mammalian target of rapamycin) approved for the treatment of patients with advanced renal cell carcinoma [28]. The mTOR signaling pathway is recognized as an important mediator in cell proliferation, growth, survival, and angiogenesis. It is the

downstream effector of the oncogenic PI3K/Akt pathway and is a key regulator of translational initiation, offering thus a range of possible combinations with other targeted therapeutics, for the treatment of solid tumors and lymphoma. More than 100 clinical trials are ongoing to identify additional malignancies that respond to temsirolimus or other mTOR inhibitors [29]. Along with the approved compound temsirolimus, there are two other mTOR inhibitors furthest along in development in oncology, everolimus and deferolimus. This class of rapamycin analogs has been known for many years. The immunosuppressant sirolimus (rapamycin, Rapamune[®]) is used in combination with other medications to prevent rejection of kidney transplants. Another derivative, tacrolimus, gained FDA approval for use in liver transplantation in 1994 and, approximately 3 years later, was approved for the prevention of acute rejection in kidney transplantation. Over the last decade tacrolimus has become the calcineurin inhibitor of choice for the prevention of rejection in renal transplantation. Everolimus is an antiproliferative agent primarily directed at cancer cells, but it is useful in other therapeutic domains as well, in particular for patients with coronary artery disease. A cobalt-chromium stent eluting everolimus was found to be more effective than a paclitaxel-eluting stent, with fewer major adverse cardiac events [30].

From the chemical point of view, these “rolimus” compounds (rapalogs) all derive from rapamycin, the macrolide antibiotic produced by *Streptomyces hygroscopicus*, initially discovered as an antifungal agent [31] and endowed with anti-inflammatory, anti-tumor and immunosuppressive properties. This *Streptomyces* strain originates from a soil sample collected in Easter island (Rapa Nui). Poorly soluble in water, sirolimus is given orally whereas temsirolimus is a water-soluble dihydroxymethyl propionic acid ester prodrug mainly used as an iv formulation. It quickly undergoes hydrolysis to sirolimus after iv administration, so that most of its clinical effects are likely attributable to the sirolimus metabolite [32].

These three examples illustrate the continued successful development of NP in oncology, prolonging thus the long history of NP-based anticancer drug discovery initiated more than 50 years ago. In each case, NP medicinal chemistry has been essential in producing desirable pharmacological properties (such as solubility, distribution or resistance to metabolism...) and structure-activity relationship (SAR) studies have been instrumental to guide drug design and to help comprehend their mechanism of action. In some cases, the unmodified NP itself can become the final product but even in this case, a robust chemistry is needed to synthesize the compound from another precursor (as in the case of trabectedin and cyanosafraicin B) and/or to establish SAR. In most cases modifications of the original NP are needed, usually rather

simple transformations of the NP to optimize formulation and ADMET properties [33]. Several other promising NP drug candidates are in the pipeline of clinical trials. These include, to cite only 3 examples, (i) vinflunine (Javlor[®] from Pierre Fabre), the latest hemisynthetic vinca alkaloid in development, prepared via super-acidic chemistry and targeting microtubules dynamics, which has revealed promising activity for the treatment of bladder, breast and non-small cell lung cancers [34,35], (ii) amrubicin (from Pharmion), a third-generation synthetic anthracycline targeting topoisomerase II, which recently received FDA fast track designation for the treatment of SCLC after first-line chemotherapy [36], (iii) alvespimycin (KOS-1022 from Kosan), an orally active heat shock protein (Hsp) 90 inhibitor derived from 17-allylaminogeldanamycin (17-AAG). The Hsp90 multichaperone complex has important roles in the development and progression of malignant transformation, promoting thus the development of Hsp90 inhibitors for cancer treatment [37].

In addition to pure compounds, NP extracts should also be considered. At this level, it is worth to mention the antitumor activity of Kanglaite injection (KLT, from Zhejiang Kianglaite Pharmaceutical Co., China), a micro-emulsion prepared from an extract of the Chinese plant *Semen coicis* [38]. In 1997, a phase III clinical study was successfully completed and the injection product was officially launched in China. KLT was later approved by the Russian Federation where it is now marketed. Kanglaite was the first drug derived from a traditional Chinese herbal remedy to go into clinical trials in the USA [39].

5. Microorganisms: the main supplier of anticancer structurally novel NP ?

For the past 40 years, plants have been a great source of secondary metabolites with extreme chemical diversity. A number of plant-derived anticancer drugs belonging to camptothecins, taxanes, vinca alkaloids and the epipodophyllotoxins families, remain extensively prescribed. But beside these well-known families, the number of plant-derived drug candidates that have reached clinical development in oncology has been reduced since 2000 (Fig. 3). Different pharmaceutical companies carry on with the development of plant NP, for examples Unibioscreen with the 2"-oxovoruscharin derivative UNBS1450 hemisynthesised from a cardenolide extracted from *Calotropis procera* [40], Pierre Fabre with a hemisynthetic triptolide derivative from the Chinese medicinal plant *Tripterygium wilfordii* or Oxigene with the microtubule-depolymerizing vascular disrupting agent combretastatin-A4 phosphate (Zybrestat[®], [41]). But overall plants now receive less attention from pharmaceutical companies, perhaps because of a (real or perceived) reduced chemo-diversity. But it is

estimated that less than 15% of higher plants have been systematically investigated for the presence of bioactive compounds [42]. Therefore, there is certainly room to explore further the plant kingdom, more deeply and perhaps differently compared to what has been done so far, using novel targets and novel methodological approaches. Despite these difficulties, several authors maintain the idea that plant NP can return to the forefront of anticancer pharmacology [43]. In the past, traditional screening approaches have allowed a rich harvest of “low hanging fruits”. Additional efforts should be devoted now to reach for the high-hanging fruits, with the help of new techniques and innovative targets.

Marine macroorganisms, another great source of secondary metabolites, have also lost some popularity in the pharmaceutical industry in recent years, mainly due to the difficulty of large scale supply. Certain companies remain very active in this domain, such as PharmaMar (Madrid, Spain) with an impressive pipeline of marine anticancer agents in development (aplidin, kahalalide F, Zalypsis,...), but the current tendency is clearly toward the exploitation of microbial sources of NP. Marine macroorganisms are difficult to collect in large scale. Moreover, it is frequent that the compound of interest initially found in the macroorganism derives in fact from a symbiotic microorganism. A typical example is that of the dolastatins first isolated from the mollusc *Dolabella auricularia* and later found to be produced by cyanobacteria [44]. This is also the case for ecteinascidin as stated above, and most likely for discodermolide, halichondrin B and bryostatin 1. A significant portion of the bioactive metabolites thought originally to be products of the source animal are often synthesized by their symbiotic microbiota [45]. Microorganisms are the most attractive source for the production of medically useful secondary metabolites. Many microorganisms can be grown to large scales in culture media, facilitating an unlimited and uninterrupted supply of the raw material needed for drug development. Actinomycetes have been one of the most investigated group of bacteria during the past 50 years. Filamentous fungi have been largely studied too. But even if these two groups have already received a major attention, there is still room for the discovery of highly potent anticancer NP in these families. It is estimated that the number of antibiotics characterized to date represents less than 5% of the total. In other words, the chemical universe produced by actinomycetes remains largely underexploited [26]. In the next ten years, this percentage should be increased significantly with the improvement of cultivation methods of prokaryote organisms and potentially the growing exploitation of metagenomic approaches for uncultivable microorganisms. New compounds belonging to novel structural classes and possessing unprecedented biological properties can be discovered

from microorganisms, whereas this is perhaps less probable with plants. In addition, the improvement of cultivation techniques together with the refinement of highly sensitive analytical methods should help the rapid dereplication of compounds [46,47]. In the near future, it is likely that the exploration of ecological niches and habitats, in particular marine environments with a large biodiversity, will furnish structurally novel NP of prime pharmacological interest. Personally, I share the view that a renaissance of drug discovery inspired by natural products can be anticipated for the next decade [48]. NP may not totally recapture the leading position they once held as a foundation for anticancer drug discovery and development but they will continue to play a major role in antitumor pharmacology and drug discovery. We already see the premises of the renaissance of NP with the approval of the aforementioned anticancer drugs ixabepilone, trabectedin and temsirolimus, and with other bacterial products such as the histone deacetylase inhibitor depsipeptide Romidepsin (FK228) which has received a fast-track status by the FDA in October 2004 as monotherapy for the treatment of patients with refractory cutaneous T-cell lymphoma, i.e. only ten years after its first isolation from *Chromobacterium violaceum* No.968 [49]. Even if the discovery of tumor-active NP remains a critical challenge and a risky option in the pharmaceutical industry, this strategy does not necessarily take longer time than chemistry-based small chemical molecules drug discovery.

6. Natural product-biological target: a *pas de deux* in drug discovery

In a *pas de deux*, the French for "step of two" in a ballet, the man plays a pivotal role by stabilizing, lifting, and turning the lady to take positions she would never be able to do on her own. A close complicity between the two partners is essential. A parallel situation occurs in drug discovery strategies where biological targets and NP-based libraries must be adapted to maximize their exploitation. NP drug discovery is guided at every turn by biological assays. Chances of discovering products of pharmacological interest depend on the quality of the NP source with a large biodiversity, adapted cultivation processes, resources of chemistry, etc. but it depends also on the quality of the targets or biological procedures set up to detect the compounds of interest. Not all molecular targets are well adapted to the identification of pharmacologically active NP. For example, the screening of natural extracts against purified protein kinases is generally difficult, with a higher percentage of false positives compared to synthetic chemical libraries. Enzyme-linked immunosorbent assays and cell-based assays are certainly better adapted to the screening of NP extracts. The design of highly robust and

sensitive high throughput screening (HTS) assays is a key element. For example, the specific design of a HTS procedure for the discovery of naturally-occurring proteasome inhibitors [50] allowed us to identify original molecules from a plants collection, such as physalin A from the tropical herb *Physalis angulata* [51].

The most popular approach to identify novel NP in oncology refers to cell cytotoxicity measurements. Cell-based assays are routinely used to evaluate the cytotoxicity of standardised extracts (or pure natural products) against murine or human cancer cells cultivated in vitro. Extract *A* tested against cell type *B* leads to the isolation of a novel cytotoxic agent *C* is a typical published story. The cell type can be varied almost indefinitely to include cancer cells of different tissue origin (breast, lung, prostate, bladder...), cell lines sensitive or resistant to a given anticancer drug or even multi-resistant, cells deficient for a specific enzyme (e.g. topoisomerase-mutated cells) or an altered pathway (e.g. cell with deficient DNA-repair), the use of non-tumoral (but often immortalised) “normal” cells, etc. These models are useful, robust and easy to set up but often too permissive. These monolayer culture models are routinely employed to identify conventional cytotoxic agents which then have to be tested in animal models, requiring long and expensive procedures. Whenever possible, this classical approach should be replaced with more sophisticated alternative screening procedures. The use of sophisticated models, more stringent and of a higher predictive value for the clinical efficacy should be favoured. An interesting approach consists to use multicellular tumor spheroid models which are of intermediate complexity between in vivo tumors and in vitro monolayer cultures. Multicellular spheroids can be generated in 96-well plates format for HTS, from a variety of tumor cell lineages [52,53]. Similar approaches can be established to study heterologous cell interactions in solid tumors and the cellular tumor environment [54]. Such 3-D culture models hold great promise to investigate the role of certain tumor initiating cells and their growth inhibition by specific natural products. It is time now to incorporate these 3-D test systems into drug development operations.

Beside cytotoxicity measurements, screening procedure for the discovery of novel NP frequently relies on the use of specific cell lines adapted or engineered to detect drug-induced apoptosis [55], cell cycle perturbations or alteration of specific molecular pathways. A typical example is that of the proteasome, a key component for limiting tumor cell proliferation and a target particularly well suited to the identification of NP. Proteasome inhibitors induce programmed cell death preferentially in transformed cells [56]. The drug bortezomib (Velcade[®] from Millenium) has been approved initially for the treatment of

relapsed/refractory multiple myeloma and more recently for the treatment of mantle cell lymphoma, for which this agent has become a standard of care. This boronic acid dipeptide is an effective reversible inhibitor of the chymotryptic protease in the 26S proteasome, which blocks activation of nuclear factor κ B, resulting in increased apoptosis, decreased angiogenic cytokine production, and inhibition of tumor cell adhesion to stroma [57]. The development of a new generation of proteasome inhibitors that hold the promise of efficacy in bortezomib-resistant tumors is an active pharmacological field. Arguably the most promising drug in this category is the chlorinated natural product salinosporamide (Nereus Pharmaceuticals) from the marine actinomycete *Salinispora tropica*. Unlike bortezomib, which is a reversible inhibitor, salinosporamide covalently binds to the proteasome, resulting in the irreversible inhibition of 20S proteasome activity. Salinosporamide was shown to act synergistically with bortezomib to trigger apoptosis in multiple myeloma cells *in vitro* and *in vivo* in a human plasmacytoma xenograft mouse model [58,59]. This natural product is active orally and has shown efficacy in a range of animal models for both hematological malignancies and solid tumors. It is currently undergoing phase I clinical trials. Salinosporamide, and the structurally-related γ -lactams omuralide and lactacystin, also illustrate the interest of exploiting microbial natural products in the search for novel anticancer agents.

Another novel promising irreversible inhibitor of the proteasome is carfilzomib (PR-171, Proteolix Inc.), derived from the actinomycete metabolite epoxomicin, which inhibits proliferation and activates apoptosis in patient-derived multiple myeloma cells. This epoxy-ketone peptidyl inhibitor shows increased efficacy compared with bortezomib and is active against cell samples from patients with clinical bortezomib resistance [60]. Carfilzomib is currently in Phase 2 clinical trials in patients with relapsed or refractory multiple myeloma and in Phase 1 in lymphoma. Other microbial products targeting the proteasome have been identified, such as the cinnabaramides isolated from a terrestrial streptomycete, belactosines A and C from *Streptomyces sp.*, the cyclic peptide TMC-95 from the fungus *Apiospora montagnei*, Fellutamide B from *Penicillium fellutanum*, and syringolin A secreted in plants by a strain of *Pseudomonas syringae* [61]. Syringolin A is a pseudo-peptide virulence factor that irreversibly inhibits all three catalytic activities of eukaryotic proteasomes, via a novel mechanism of covalent binding to the catalytic subunits [62]. The α,β -unsaturated carbonyl of syringolin A reacts with a threonine residue of the proteasome to form a covalent bond and this cross-linking is considered at the origin of the antiproliferative and pro-apoptotic effects observed in ovarian and neuroblastoma cancer cells [63]. Proteasome inhibitors have also been identified from plant extracts. This is the case for celastrol from the Chinese medicinal

plant *Tripterygium wilfordii*, withaferin A from the medicinal plant "Indian Winter Cherry", pristimerin from plants of the Celastraceae and Hippocrateaceae families, and physalin A from the plant *Physalis angulata*, as mentioned above. But of course, this field is not restricted to natural products and promising synthetic molecules have been reported as well, such as the phenylpyridine-containing boronic acid derivative CEP-18770 which is a novel orally-active inhibitor of the chymotrypsin-like activity [64,65]. These proteasome inhibitors are primarily designed as anticancer agents but they can be of interest for other pathologies characterized by alterations in the proteasome proteolytic pathway including autoimmune and inflammatory diseases, myocardial infarction, and ischemic brain injury [66]. The use of proteasome inhibitors has been suggested for the treatment of rheumatoid arthritis. In this case, the rational is to limit the growth of fibroblast-like synoviocytes that mediate joint destruction [67].

Many new targets have been discovered and biological/biochemical tools such as sh/siRNA or engineered cellular and animal models are useful to validate these targets. But in parallel, the full potential of old targets should be exploited further. Just to cite one example, topoisomerase I is a well-known target in oncology but thus far there is only one chemical series of inhibitors on the market: the camptothecins (Fig. 4). Topotecan, irinotecan, and newer compounds in clinical trials such as gimatecan and diflomotecan all exploit the same property of the enzyme, DNA cleavage which they promote through stabilization of the DNA-topoisomerase I covalent complex which is normally transient. There is room for more potent, better tolerated and orally active inhibitors acting at this level, such as the indolocarbazole edotecarin, the indenoisoquinoline derivative NSC 724998 and the dibenzonaphthyridinone ARC-111 for examples [68]. But there are also opportunities to target other activities of this key enzyme for tumor cell proliferation. Topoisomerase I interacts with a large number of specific proteins important for the control of transcription and DNA repair, such as the coactivator of p53 topors, nucleolin, the tumor suppressor proteins prostate apoptosis response-4 (Par-4) and NKX3.1, SV40 large T antigen and certain helicases (Fig. 4). These protein interfaces may be targeted, if suitable assays can be developed. Topoisomerase I also carries a kinase activity important for the regulation of RNA splicing [69]. Although the link between the topoI kinase activity and cancer is still poorly understood, it is nevertheless clear that this is a potential new area for pharmacological intervention. In addition to its well-known DNA relaxation activity, human topoisomerase I is responsible for the phosphorylation of Ser/Arg-rich (SR) splicing proteins, in particular the splicing factor

2/alternative splicing factor. SF2/ASF and topoisomerase I regulate their activity via an interaction between two closely-spaced RNA recognition motifs of the splicing factor and the N-terminal cap region of topoisomerase I which is essential for the kinase reaction [70]. This mechanism can be modulated with NP and derivatives such the glycosylated indolocarbazole derivative NB-506, derived from the streptomycete metabolite BE-13793C and from rebeccamycin produced by the actinomycete *Saccharothrix aerocolonigenes*. NB-506 acts as a potent inhibitor of both kinase and relaxing activities of topoisomerase I (Fig. 4), and modulates pre-mRNA splicing through inhibition of SF2/ASF phosphorylation [71]. SF2/ASF is as a splicing factor and also mediates postsplicing activities such as mRNA export and translation initiation of bound mRNAs, through interactions between SF2/ASF with both the mTOR kinase and the phosphatase PP2A. SF2/ASF functions as an adaptor protein to recruit signaling molecules responsible for regulation of translation of specific mRNAs [72]. The phosphorylation state of the SR domain influences the role of SF2/ASF in cytoplasmic RNA processing. A model has been proposed whereby reversible protein phosphorylation differentially regulates the subcellular localization and activity of shuttling SR proteins [73]. Moreover, recent studies have revealed that SF2/ASF is up-regulated in various human tumors and can act as an oncoprotein [74]. Therefore it might be valuable to try to manipulate SF2/ASF-mediated splicing via an interference with the kinase activity of topoisomerase I. The same approach can be envisaged with other kinases involved in the regulation of SF2/ASF-dependent splicing such as Cdc2-like kinase-1 (Clk-1) [75]. There are multiple pharmacological options to modulate the activity of SR splicing factors and to try to control splicing [76]. There is an estimated 100,000 splice variants of messenger RNA transcripts from the approximately 25,000 genes estimated in humans. This area of pharmacology is practically a desert.

Novel targets including isolated molecular receptors and more complex phenotypic signals, and old targets revisited offer multiple perspectives to exploit differently NP and bio-extracts. In oncology, the knowledge of disease-specific molecular alterations is essential. The therapeutic value of a given target is proportional to the quantification of the role of this target in well-defined tumor types, when the druggability of a given target has been properly assessed and when its full clinical potential has been defined in terms of safety and efficacy. An original NP with a novel mechanism of action has *de facto* more chances to be pushed into development than a NP directed at a conventional target. In the current era of targeted therapy, it is a challenge to develop an anticancer drug, even a structurally new NP, whose

mechanism of action resembles that of classical molecules, in particular DNA-interacting drugs, topoisomerase inhibitors or tubulin/microtubule-interacting agents. But a brand new NP “in advance of its time”, i.e. with a target unknown or poorly characterized at the pharmacological level, can be difficult to manipulate as well. A well defined molecular target is not always absolutely mandatory, but it is highly preferable [33].

7. Conclusion

The impact of NP on anticancer drug discovery has been very high in the past century, it was certainly more limited for the past 8 years but will remain considerable for many more years, not only for cancer but also for diseases such as microbial and parasitic infections [4]. The approval of the aforementioned drugs ixabepilone, trabectedin and temsirolimus is emblematic of the evolution of the field of NP in oncology. The following points are important:

- The field is alive and well in academia but needs more supports in pharmaceutical companies and new NP-derived molecules in clinical trials.
- Conventional cytotoxic agents remain present and expected from clinicians, but with a preference for NP targeting pathways other than the tubulin/microtubule network or the DNA replication machinery, considered old-fashioned. There is an unquestionable demand for novel cytotoxic agents bringing not only gains in efficacy but decreasing the frequency of adverse events and offering a better convenience in administration [77]. In this sense, ixabepilone pursues the long established tradition of cytotoxic NP directed at microtubules. The fact that this compound originates from a microorganism, and not a plant as for the taxanes or vinca alkaloids, illustrates the increasing importance of microbial sources for the discovery of medically useful NP. Plants remains a sure value but cultivated microorganisms will certainly play a dominant role in the future.
- The development of marine NP is progressing actively. A marine microorganism has provided a new tumor-active drug (Trabectedin) now hemi-synthesized from a natural precursor (cyanosafracin B) extracted from a microorganism. The shift from macro to micro-organisms illustrates the evolution of the field.

- The detailed understanding of the mechanism of action of a NP is essential. Trabectedin is more than a classical DNA-alkylating agent; the downstream implication of the DNA repair machinery is critical for the drug action and possibly for patients selection.
- We have entered the era of targeted therapy with NP. This is probably the most salient feature of the field and a decisive step toward the demonstration that NP can continue to play a key role in modern antitumor pharmacology. The approval of temsirolimus, likely followed by one or two structurally related mTOR inhibitors in the near future, marks the transition between conventional chemotherapy with highly cytotoxic NP and more selective microbial products targeting a specific molecular pathway essential for the proliferation of cancer cells. Certainly in this particular case the chemotype is not new (everolimus has been known for years) but the first hurdle has been crossed to show that it makes sense to consider NP as a valuable source for innovative targeted therapeutic agents. During years 2000-2006, NP have been somewhat neglected and arguably underestimated by most pharmaceutical companies, to favor biologics and synthetic small molecules targeting a narrow ATP-pocket of protein kinases in particular. Nevertheless, during the same period the US approval success rate has remained low, around 8% for oncology [78] and the medical need is always high among cancer patients and their clinicians. The approval of the 3 new NP-derived drugs in 2007 hopefully announces a new departure for NP-based drug discovery in oncology. We are ready for this long awaited comeback.

Acknowledgments

The author thanks many colleagues from the IRPF and PFO for fruitful discussions on natural products and reading of the manuscript.

References

- [1] Carter SK, Friedman MA. 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC, DIC, NSC-45388)--a new antitumor agent with activity against malignant melanoma. *Eur J Cancer* 1972;8:85-92.
- [2] Benson AB 3rd. Epidemiology, disease progression, and economic burden of colorectal cancer. *J Manag Care Pharm.* 2007;13(Suppl C):S5-18.
- [3] Butler MS. Natural products to drugs: natural product-derived compounds in clinical trials. *Nat Prod Rep* 2008;25:475-516.
- [4] Butler MS, Newman DJ. Mother Nature's gifts to diseases of man: the impact of natural products on anti-infective, anticholesteremics and anticancer drug discovery. *Prog Drug Res* 2008;65:3-44.
- [5] Blum RH, Carter SK. Adriamycin. A new anticancer drug with significant clinical activity. *Ann Intern Med* 1974;80:249-259.
- [6] Hata T, Hoshi T, Kanamori K, Matsumae A, Sano Y, Shima T, Sugawara R. Mitomycin, a new antibiotic from *Streptomyces*. I. *J Antibiot (Tokyo)* 1956;9:141-146.
- [7] Gradishar W, Cortes J. Clinical efficacy and emerging therapeutic utilization of novel taxanes. *Eur J Cancer* 2008,Suppl6:12-21.
- [8] Aapro MS, Von Minckwitz G. Molecular basis for the development of novel taxanes in the treatment of metastatic breast cancer. *Eur J Cancer* 2008,Suppl6:3-11.
- [9] Scripture CD, Figg WD, Sparreboom A. Paclitaxel chemotherapy: from empiricism to a mechanism-based formulation strategy. *Ther Clin Risk Manag* 2005;1:107-114.
- [10] Hawkins MJ, Soon-Shiong P, Desai N. Protein nanoparticles as drug carriers in clinical medicine. *Adv Drug Deliv Rev* 2008;60:876-885.
- [11] Wong J, Brugger A, Khare A, Chaubal M, Papadopoulos P, Rabinow B, Kipp J, Ning J. Suspensions for intravenous (IV) injection: A review of development, preclinical and clinical aspects. *Adv Drug Deliv Rev* 2008;60:939-954.
- [12] Nicum SJ, O'Brien ME. Topotecan for the treatment of small-cell lung cancer. *Expert Rev Anticancer Ther* 2007;7:795-801.
- [13] Marty M, Fumoleau P, Adenis A, Rousseau Y, Merrouche Y, Robinet G, Senac I., Puozzo C. Oral vinorelbine pharmacokinetics and absolute bioavailability study in patients with solid tumors, *Ann Oncol* 2001,12:1643-1649.
- [14] Jassem J, Ramlau R, Karnicka-Mlodkowska H, Krawczyk K, Krzakowski M, Zatloukal P, Lemarié E, Hartmann W, Novakova L, O'Brien M, Depierre A. A multicenter randomized phase II study of oral vs. intravenous vinorelbine in advanced non-small-cell lung cancer patients. *Ann Oncol* 2001;12:1375-1381.
- [15] Aisner J. Overview of the changing paradigm in cancer treatment: oral chemotherapy, *Am J Health Syst Pharm* 2007;64(Suppl.5):S4-S7.

- [16] Jensen LH, Osterlind K, Rytter C. Randomized cross-over study of patient preference for oral or intravenous vinorelbine in combination with carboplatin in the treatment of advanced NSCLC. *Lung Cancer* 2008;in press.
- [17] Findlay M, von Minckwitz G, Wardley A. Effective oral chemotherapy for breast cancer: pillars of strength. *Ann Oncol* 2008;19:212-222.
- [18] Bollag DM, McQueney PA, Zhu J, Hensens O, Koupal L, Liesch J, Goetz M, Lazarides E, Woods CM. Etoposides, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. *Cancer Res* 1995;55:2325-2333.
- [19] Fornier MN. Ixabepilone, first in a new class of antineoplastic agents: the natural etoposides and their analogues. *Clin Breast Cancer* 2007;7:757-763.
- [20] Lee FY, Borzilleri R, Fairchild CR, Kamath A, Smykla R, Kramer R, Vite G. Preclinical discovery of ixabepilone, a highly active antineoplastic agent. *Cancer Chemother Pharmacol* 2008;in press.
- [21] Lee FY, Smykla R, Johnston K, Menard K, McGlinchey K, Peterson RW, Wiebesiek A, Vite G, Fairchild CR, Kramer R. Preclinical efficacy spectrum and pharmacokinetics of ixabepilone. *Cancer Chemother Pharmacol* 2008;in press.
- [22] Altmann KH, Memmert K. Etoposides as lead structures for new anticancer drugs--pharmacology, fermentation, and structure-activity-relationships. *Prog Drug Res* 2008;66:275-334.
- [23] Soares DG, Escargueil AE, Poindessous V, Sarasin A, de Gramont A, Bonatto D, Henriques JA, Larsen AK. Replication and homologous recombination repair regulate DNA double-strand break formation by the antitumor alkylator ecteinascidin 743. *Proc Natl Acad Sci USA* 2007;104:13062-13067.
- [24] Carter NJ, Keam SJ. Trabectedin : a review of its use in the management of soft tissue sarcoma and ovarian cancer. *Drugs* 2007;67:2257-2276.
- [25] Simmons TL, Andrianasolo E, McPhail K, Flatt P, Gerwick WH. Marine natural products as anticancer drugs. *Mol Cancer Ther* 2005;4:333-342.
- [26] Singh SB, Pelaez F. Biodiversity, chemical diversity and drug discovery. *Prog Drug Res* 2008;65:143-174.
- [27] Mayer AMS, Gustafson KR. Marine pharmacology in 2005–2006: Antitumour and cytotoxic compounds. *Eur J cancer* 2008;44:2357-2387.
- [28] Ma WW, Jimeno A. Temsirolimus. *Drugs Today* 2007;43:659-669
- [29] Figlin RA. Mechanisms of disease: survival benefit of temsirolimus validates a role for mTOR in the management of advanced RCC. *Nat Clin Pract Oncol* 2008;in press.
- [30] Stone GW, Midei M, Newman W, Sanz M, Hermiller JB, Williams J, Farhat N, Mahaffey KW, Cutlip DE, Fitzgerald PJ, Sood P, Su X, Lansky AJ; SPIRIT III Investigators. Comparison of an everolimus-eluting stent and a paclitaxel-eluting stent

- in patients with coronary artery disease: a randomized trial. *JAMA* 2008;299:1903-1913.
- [31] Vezina C, Kudelski A, Sehgal SN. Rapamycin (AY 22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiotics* 1975;28:721-726.
- [32] Hartford CM, Ratain MJ. Rapamycin: something old, something new, sometimes borrowed and now renewed. *Clin Pharmacol Ther* 2007;82:381-388.
- [33] Ganesan A. The impact of natural products upon modern drug discovery. *Curr Opin Chem Biol* 2008;12:306-317.
- [34] Fahy J, Hellier P, Breillout F, Bailly C. Vinflunine: discovery and synthesis of a novel microtubule inhibitor. *Semin Oncol* 2008;35(Suppl.3):S3-5.
- [35] Bellmunt J, Delgado FM, George C. Clinical activity of vinflunine in transitional cell carcinoma of the urothelium and other solid tumors. *Semin Oncol* 2008;35(Suppl.3):S34-43.
- [36] Kurata T, Okamoto I, Tamura K, Fukuoka M. Amrubicin for non-small-cell lung cancer and small-cell lung cancer. *Invest New Drugs* 2007;25:499-504.
- [37] Pearl LH, Prodromou C, Workman P. The Hsp90 molecular chaperone: an open and shut case for treatment. *Biochem J* 2008;410:439-453.
- [38] Lu Y, Li C-S, Dong Q. Chinese herb related molecules of cancer-cell-apoptosis: a minireview of progress between Kanglaite injection and related genes. *J Exp Clin Cancer Res.* 2008;27:31.
- [39] Normile D. Asian medicine: the new face of traditional Chinese medicine. *Science* 2003;299:188-190.
- [40] Mijatovic T, De Nève N, Gailly P, Mathieu V, Haibe-Kains B, Bontempi G, Lapeira J, Decaestecker C, Facchini V, Kiss R. Nucleolus and c-Myc: potential targets of cardenolide-mediated antitumor activity. *Mol Cancer Ther* 2008;7:1285-1296.
- [41] Petit I, Karajannis MA, Vincent L, Young L, Butler J, Hooper AT, Shido K, Steller H, Chaplin DJ, Feldman E, Rafii S. The microtubule-targeting agent CA4P regresses leukemic xenografts by disrupting interaction with vascular cells and mitochondrial-dependent cell death. *Blood* 2008;111:1951-1961.
- [42] Pieters L, Vlietinck AJ. Bioguided isolation of pharmacologically active plant components, still a valuable strategy for the finding of new lead compounds? *J Ethnopharmacol* 2005;100 :57-60.
- [43] McChesney JD, Venkataraman SK, Henri JT. Plant natural products: back to the future or into extinction? *Phytochemistry* 2007;68:2015-2022.
- [44] Sielaff H, Christiansen G, Schwecke T. Natural products from cyanobacteria: Exploiting a new source for drug discovery. *IDrugs* 2006;9:119-127.

- [45] Simmons TL, Coates RC, Clark BR, Engene N, Gonzalez D, Esquenazi E, Dorrestein PC, Gerwick WH. Biosynthetic origin of natural products isolated from marine microorganism-invertebrate assemblages. *Proc Natl Acad Sci USA* 2008;105:4587-4594.
- [46] Lang G, Mayhudin NA, Mitova MI, Sun L, van der Sar S, Blunt JW, Cole AL, Ellis G, Laatsch H, Munro MH. Evolving trends in the dereplication of natural product extracts: new methodology for rapid, small-scale investigation of natural product extracts. *J Nat Prod* 2008;in press.
- [47] Koehn FE. High impact technologies for natural products screening. *Prog Drug Res* 2008;65:177-210.
- [48] Harvey AL. Natural products as a screening resource. *Curr Opin Chem Biol* 2007;11:480-484.
- [49] Masuoka Y, Shindoh N, Inamura N. Histone deacetylase inhibitors from microorganisms: the Astellas experience. *Prog Drug Res* 2008;66:335, 337-59.
- [50] Ausseil F, Samson A, Aussagues Y, Vandenberghe I, Créancier L, Pouny I, Kruczynski A, Massiot G, Bailly C. High-throughput bioluminescence screening of ubiquitin-proteasome pathway inhibitors from chemical and natural sources. *J Biomol Screen* 2007;12:106-116.
- [51] Vandenberghe I, Créancier L, Vispé S, Annereau JP, Barret JM, Pouny I, Samson A, Aussagues Y, Massiot G, Ausseil F, Bailly C, Kruczynski A. Physalin B, a novel inhibitor of the ubiquitin-proteasome pathway, triggers NOXA-associated apoptosis. *Biochem Pharmacol* 2008;76:453-462.
- [52] Ivascu A, Kubbies M. Rapid generation of single-tumor spheroids for high-throughput cell function and toxicity analysis. *J Biomol Screen* 2006;11:922-932.
- [53] Herrmann R, Fayad W, Schwarz S, Berndtsson M, Linder S. Screening for compounds that induce apoptosis of cancer cells grown as multicellular spheroids. *J Biomol Screen* 2008;13:1-8.
- [54] Friedrich J, Ebner R, Kunz-Schughart LA. Experimental anti-tumor therapy in 3-D: spheroids-old hat or new challenge? *Int J Radiat Biol* 2007;83:849-871.
- [55] Tian H, Ip L, Luo H, Chang DC, Luo KQ. A high throughput drug screen based on fluorescence resonance energy transfer (FRET) for anticancer activity of compounds from herbal medicine. *Br J Pharmacol* 2007;150:321-334.
- [56] Orłowski RZ, Kuhn DJ. Proteasome inhibitors in cancer therapy: lessons from the first decade. *Clin Cancer Res* 2008;14:1649-57
- [57] Roccaro AM, Vacca A, Ribatti D. Bortezomib in the treatment of cancer. *Recent Patents Anticancer Drug Discov* 2006;1:397-403.
- [58] Chauhan D, Catley L, Li G, Podar K, Hideshima T, Velankar M, Mitsiades C, Mitsiades N, Yasui H, Letai A, Ovaia H, Berkers C, Nicholson B, Chao TH, Neuteboom ST, Richardson P, Palladino MA, Anderson KC. A novel orally active proteasome inhibitor

- induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. *Cancer Cell* 2005;8:407-419.
- [59] Chauhan D, Singh A, Brahmandam M, Podar K, Hideshima T, Richardson P, Munshi N, Palladino MA, Anderson KC. Combination of proteasome inhibitors bortezomib and NPI-0052 trigger in vivo synergistic cytotoxicity in multiple myeloma. *Blood* 2008;111:1654-1664.
- [60] Kuhn DJ, Chen Q, Voorhees PM, Strader JS, Shenk KD, Sun CM, Demo SD, Bennett MK, van Leeuwen FW, Chanan-Khan AA, Orlowski RZ. Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. *Blood* 2007;110:3281-3290.
- [61] Moore BS, Eustáquio AS, McGlinchey RP. Advances in and applications of proteasome inhibitors. *Curr Opin Chem Biol* 2008;12:434-440.
- [62] Groll M, Schellenberg B, Bachmann AS, Archer CR, Huber R, Powell TK, Lindow S, Kaiser M, Dudler R. A plant pathogen virulence factor inhibits the eukaryotic proteasome by a novel mechanism. *Nature* 2008;452:755-758.
- [63] Coleman CS, Rocetes JP, Park DJ, Wallick CJ, Warn-Cramer BJ, Michel K, Dudler R, Bachmann AS. Syringolin A, a new plant elicitor from the phytopathogenic bacterium *Pseudomonas syringae* pv. *syringae*, inhibits the proliferation of neuroblastoma and ovarian cancer cells and induces apoptosis. *Cell Prolif* 2006;39:599-609.
- [64] Dorsey BD, Iqbal M, Chatterjee S, Menta E, Bernardini R, Bernareggi A, Cassarà PG, D'Arasmo G, Ferretti E, De Munari S, Oliva A, Pezzoni G, Allievi C, Strepponi I, Ruggeri B, Ator MA, Williams M, Mallamo JP. (2008) Discovery of a potent, selective, and orally active proteasome inhibitor for the treatment of cancer. *J Med Chem* 2008;51:1068-1072.
- [65] Piva R, Ruggeri B, Williams M, Costa G, Tamagno I, Ferrero D, Gai V, Coscia M, Peola S, Massaia M, Pezzoni G, Allievi C, Pescalli N, Cassin M, di Giovine S, Nicoli P, de Feudis P, Strepponi I, Roato I, Ferracini R, Bussolati B, Camussi G, Jones-Bolin S, Hunter K, Zhao H, Neri A, Palumbo A, Berkers C, Ovaa H, Bernareggi A, Inghirami G (2008) CEP-18770: A novel, orally active proteasome inhibitor with a tumor-selective pharmacologic profile competitive with bortezomib. *Blood* 2008;111:2765-2775.
- [66] Shah IM, Di Napoli M. The ubiquitin-proteasome system and proteasome inhibitors in central nervous system diseases. *Cardiovasc Hematol Disord Drug Targets* 2007;7:250-273.
- [67] Brun, J. Proteasome inhibition as a novel therapy in treating rheumatoid arthritis. *Med. Hypotheses* 2008;71:65-72.
- [68] Teicher BA. Next generation topoisomerase I inhibitors: Rationale and biomarker strategies. *Biochem Pharmacol* 2008;75:1262-1271.
- [69] Rossi F, Labourier E, Forné T, Divita G, Derancourt J, Riou JF, Antoine E, Cathala G, Brunel C, Tazi J. Specific phosphorylation of SR proteins by mammalian DNA topoisomerase I. *Nature* 1996;381:80-82.

- [70] Trzcińska-Daneluti AM, Górecki A, Czuby A, Kowalska-Loth B, Girstun A, Murawska M, Lesyng B, Staroń K. RRM proteins interacting with the cap region of topoisomerase I. *J Mol Biol.* 2007;369:1098-1112.
- [71] Pilch B, Allemand E, Facompré M, Bailly C, Riou JF, Soret J, Tazi J. Specific inhibition of serine- and arginine-rich splicing factors phosphorylation, spliceosome assembly, and splicing by the antitumor drug NB-506. *Cancer Res* 2001;61:6876-6884.
- [72] Michlewski G, Sanford JR, Cáceres JF. The splicing factor SF2/ASF regulates translation initiation by enhancing phosphorylation of 4E-BP1. *Mol Cell* 2008;30:179-189.
- [73] Sanford JR, Ellis JD, Cazalla D, Cáceres JF. Reversible phosphorylation differentially affects nuclear and cytoplasmic functions of splicing factor 2/alternative splicing factor. *Proc Natl Acad Sci USA* 2005;102:15042-15047.
- [74] Karni R, de Stanchina E, Lowe SW, Sinha R, Mu D, Krainer AR. The gene encoding the splicing factor SF2/ASF is a proto-oncogene. *Nat Struct Mol Biol* 2007;14:185-193.
- [75] Muraki M, Ohkawara B, Hosoya T, Onogi H, Koizumi J, Koizumi T, Sumi K, Yomoda J, Murray MV, Kimura H, Furuichi K, Shibuya H, Krainer AR, Suzuki M, Hagiwara M. Manipulation of alternative splicing by a newly developed inhibitor of Clks. *J Biol Chem* 2004;279:24246-24254.
- [76] Soret J, Gabut M, Tazi J. SR proteins as potential targets for therapy. *Prog Mol Subcell Biol* 2006;44:65-87.
- [77] Ismael GF, Rosa DD, Mano MS, Awada A. Novel cytotoxic drugs: old challenges, new solutions. *Cancer Treat Rev* 2008;34:81-91.
- [78] Reichert JM, Wenger, JB. Development trends for new cancer therapeutics and vaccines. *Drug Discov Today* 2008;13:30-37.

Figures Legends

Fig. 1. Four categories of drugs used for the treatment of cancers during the past 50 years. Cytotoxic natural products played a major role in conventional chemotherapy until the late 1990s with the advent of molecular targeted therapies using monoclonal antibodies and small molecule kinase inhibitors.

Fig. 2. The semi-synthetic vinca alkaloid vinorelbine, synthesized from the leaves of *Catharanthus roseus*, was first approved in 1989 in Europe for the i.v. treatment of non-small cell lung cancer. In 2001, the drug was approved as soft gelatin capsules for oral treatments of NSCLC.

Fig. 3. After a decade in the wilderness, in 2007 natural products make a comeback in oncology. Drugs recently approved for the treatment of cancers (year of first FDA approval) by category of origin. Note that, for the sake of clarity, all small molecules (e.g. aromatase inhibitors) are not mentioned.

Fig. 4. The multifunctionality of topoisomerase I and its pharmacological control. topoisomerase I carries three major biochemical functions: (i) control of DNA topology, via DNA cleavage and religation, an essential mechanism which can be altered by suppressors and poisons such as the clinically-used camptothecins, (ii) interaction with protein partners, including pro-apoptotic factors and (iii) regulation of RNA splicing via a kinase activity. The NP-derived antitumor drug NB-506 acts as a potent inhibitor of both kinase and relaxing activities of topoisomerase I, and modulates pre-mRNA splicing through inhibition of phosphorylation of the splicing factor SF2/ASF which is up-regulated in various human tumors. See text for more details.

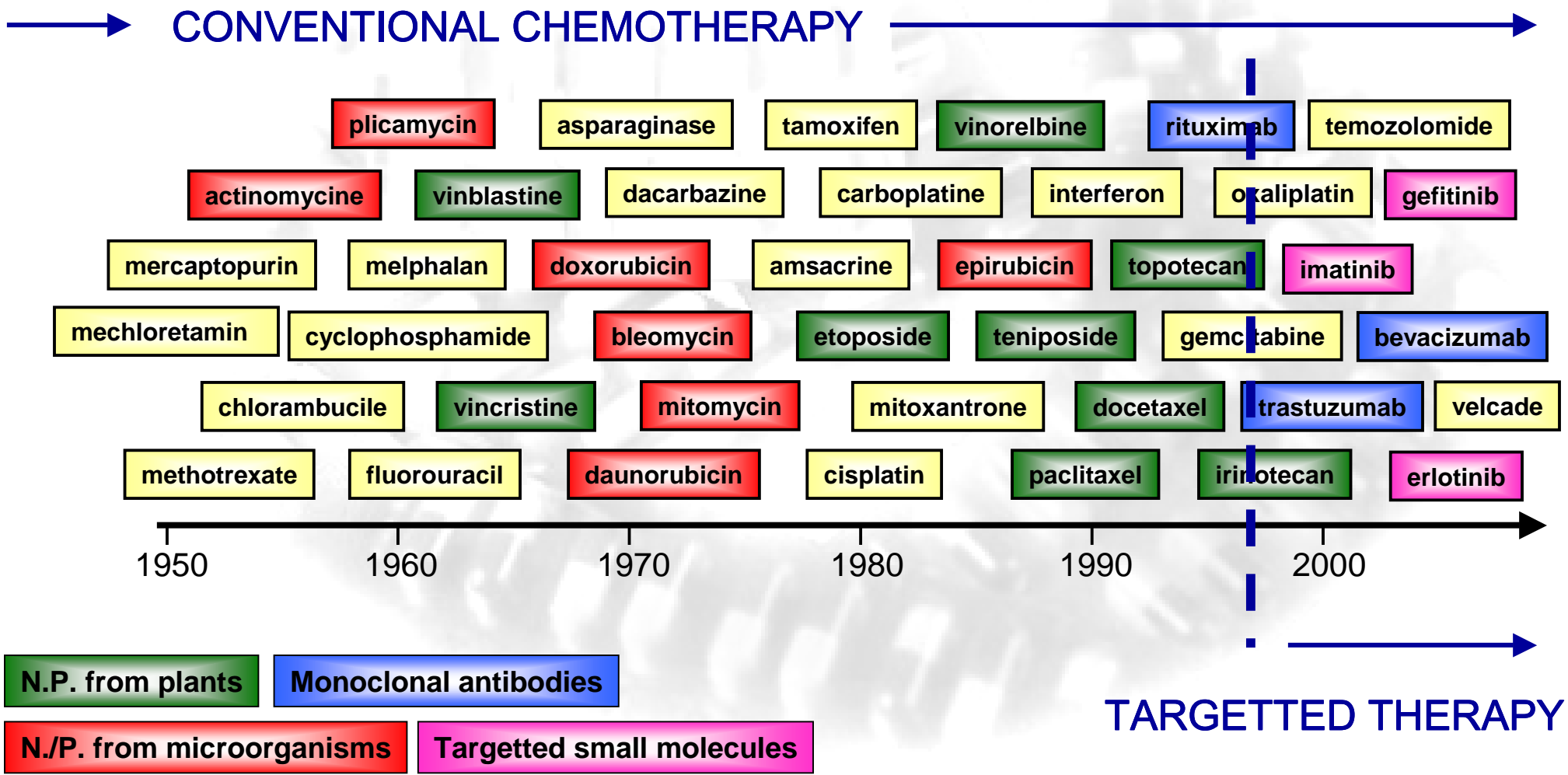


Figure 1

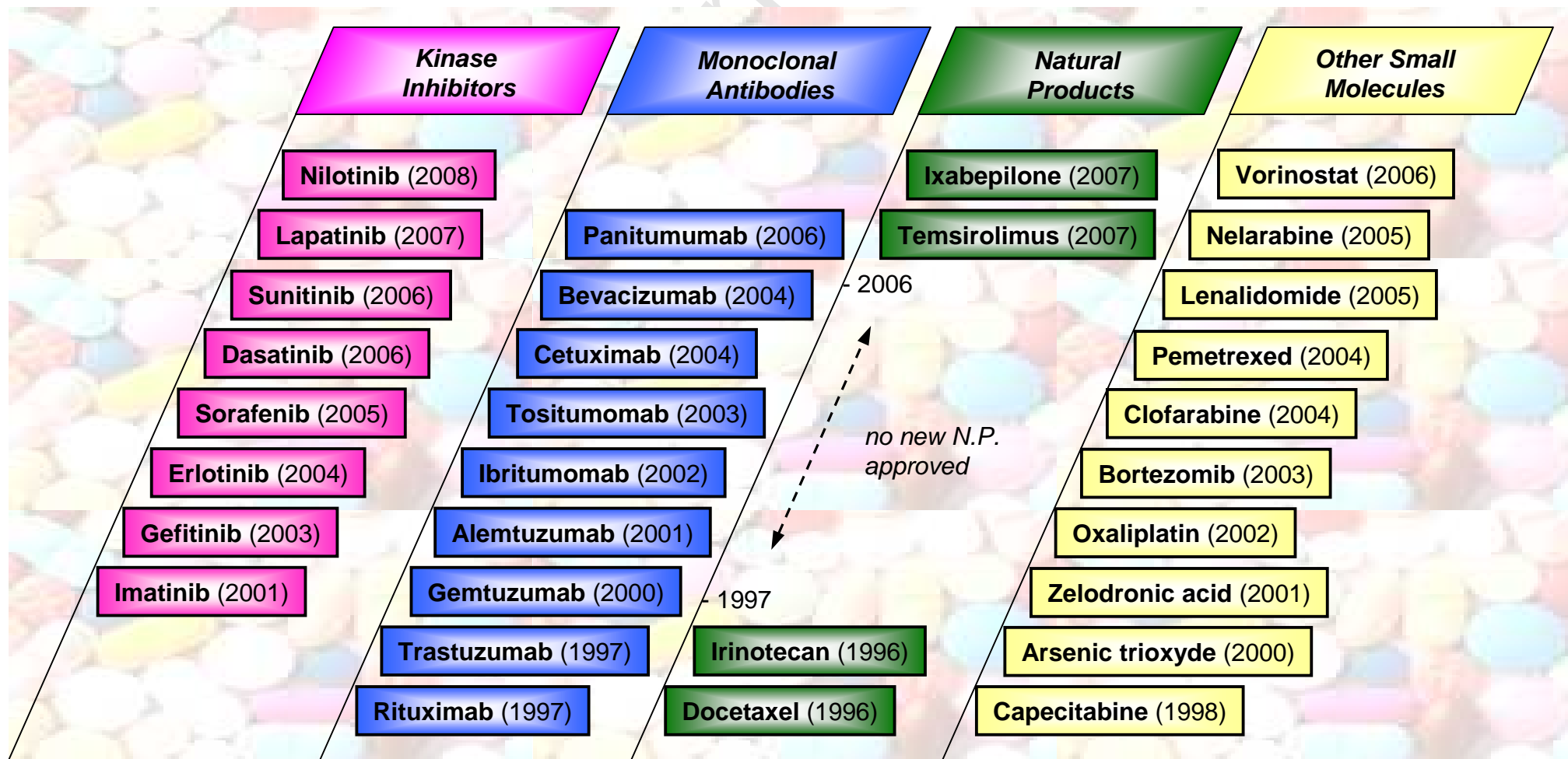


Figure 3

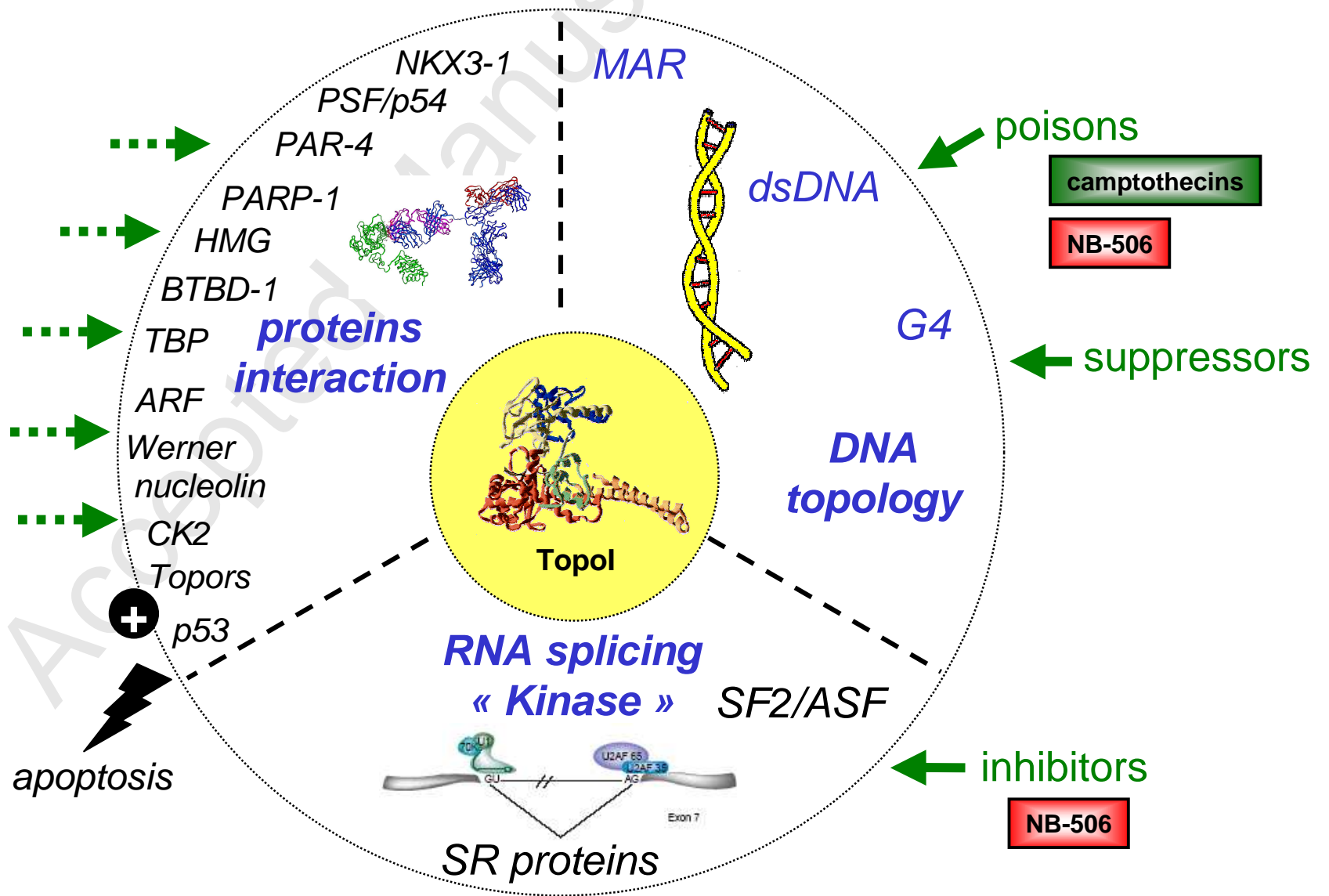


Figure 4

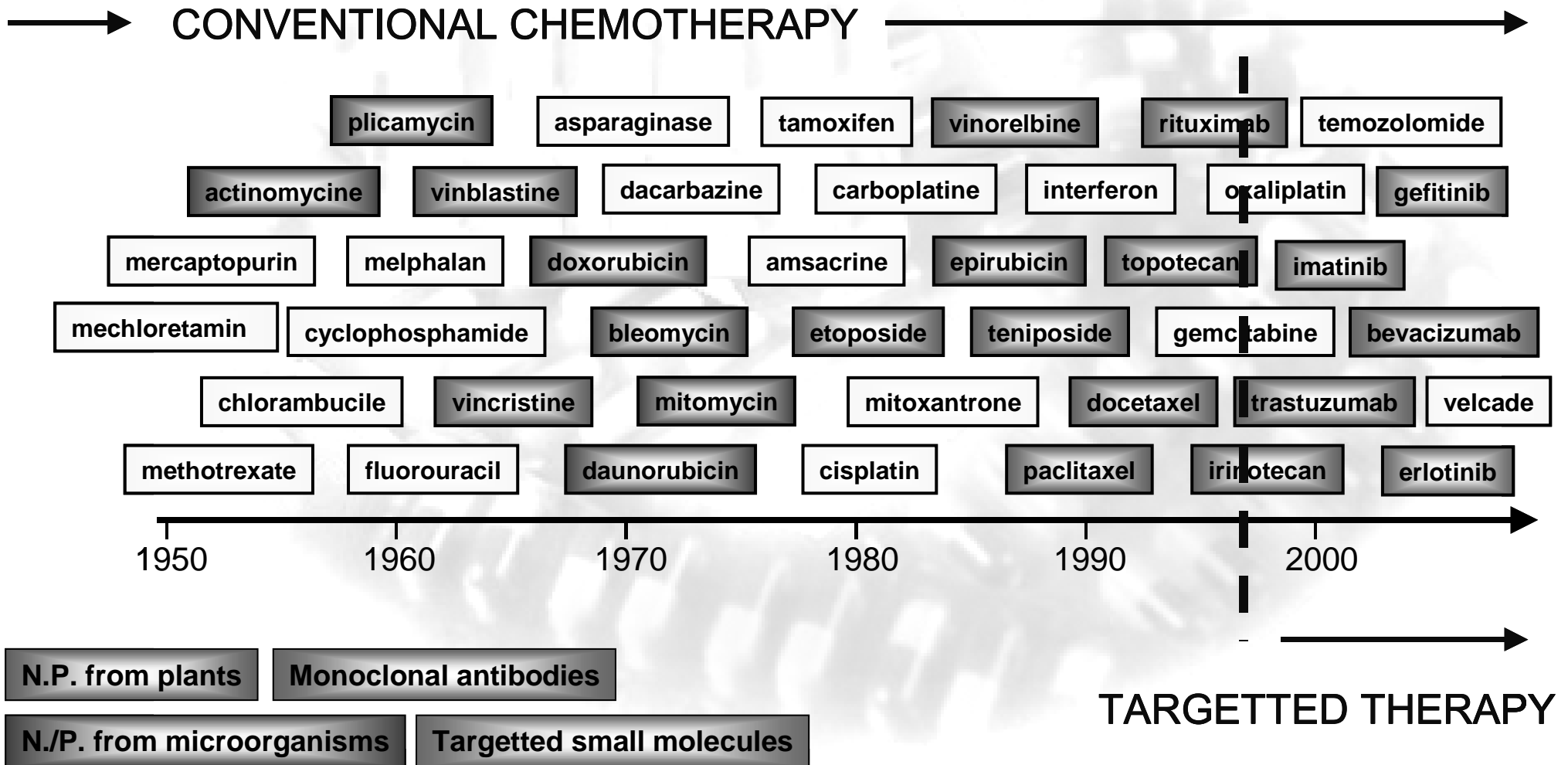
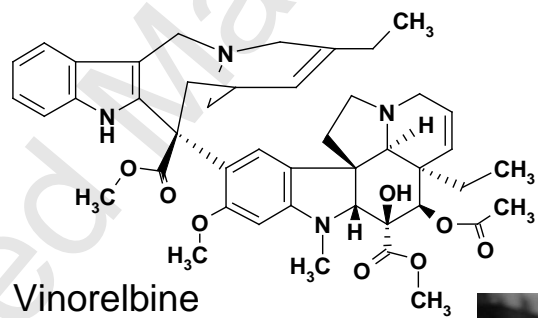


Figure 1

Accepted Manuscript



2001: oral formulation



1989: i.v. formulation



Figure 2

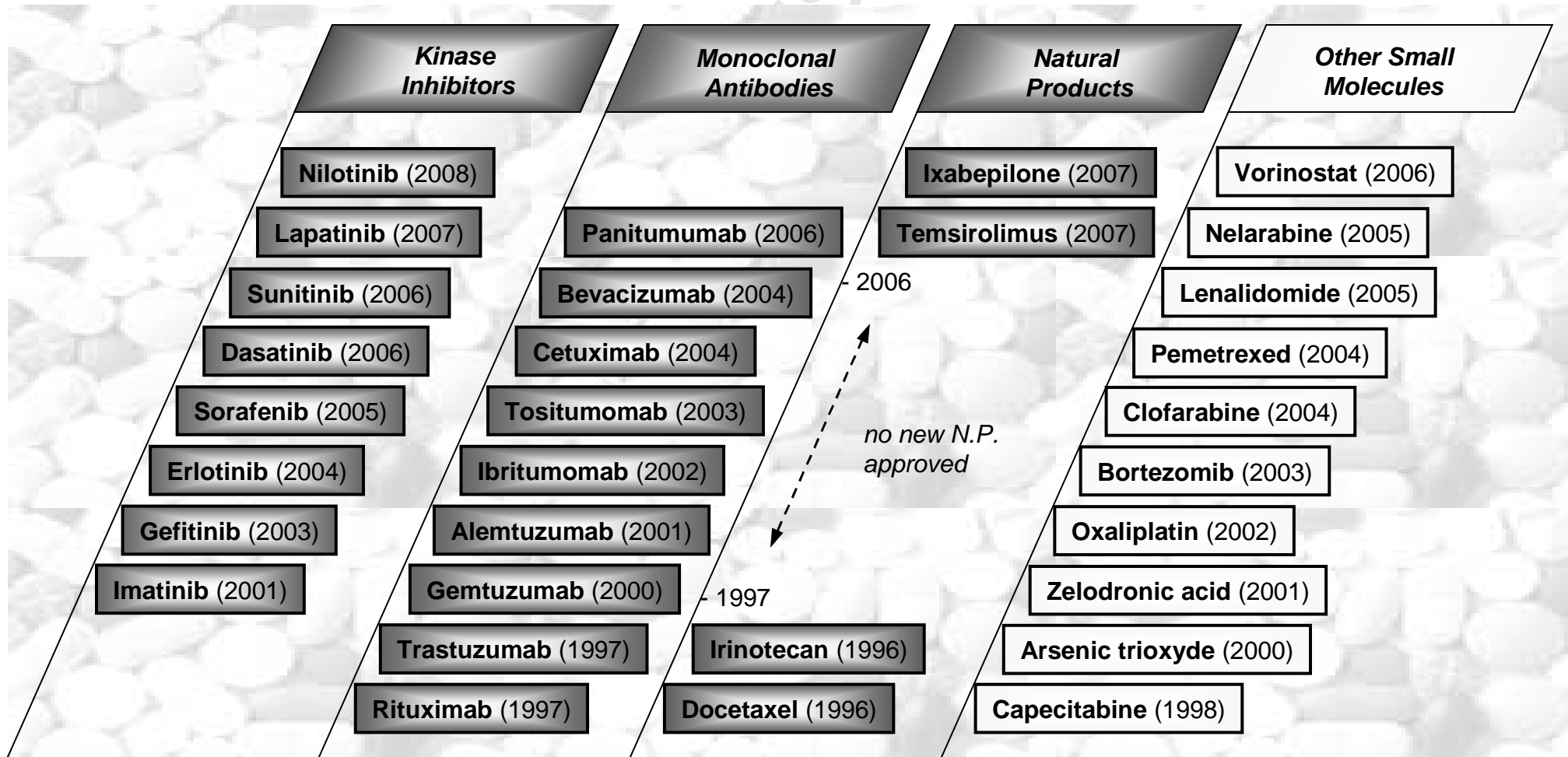


Figure 3

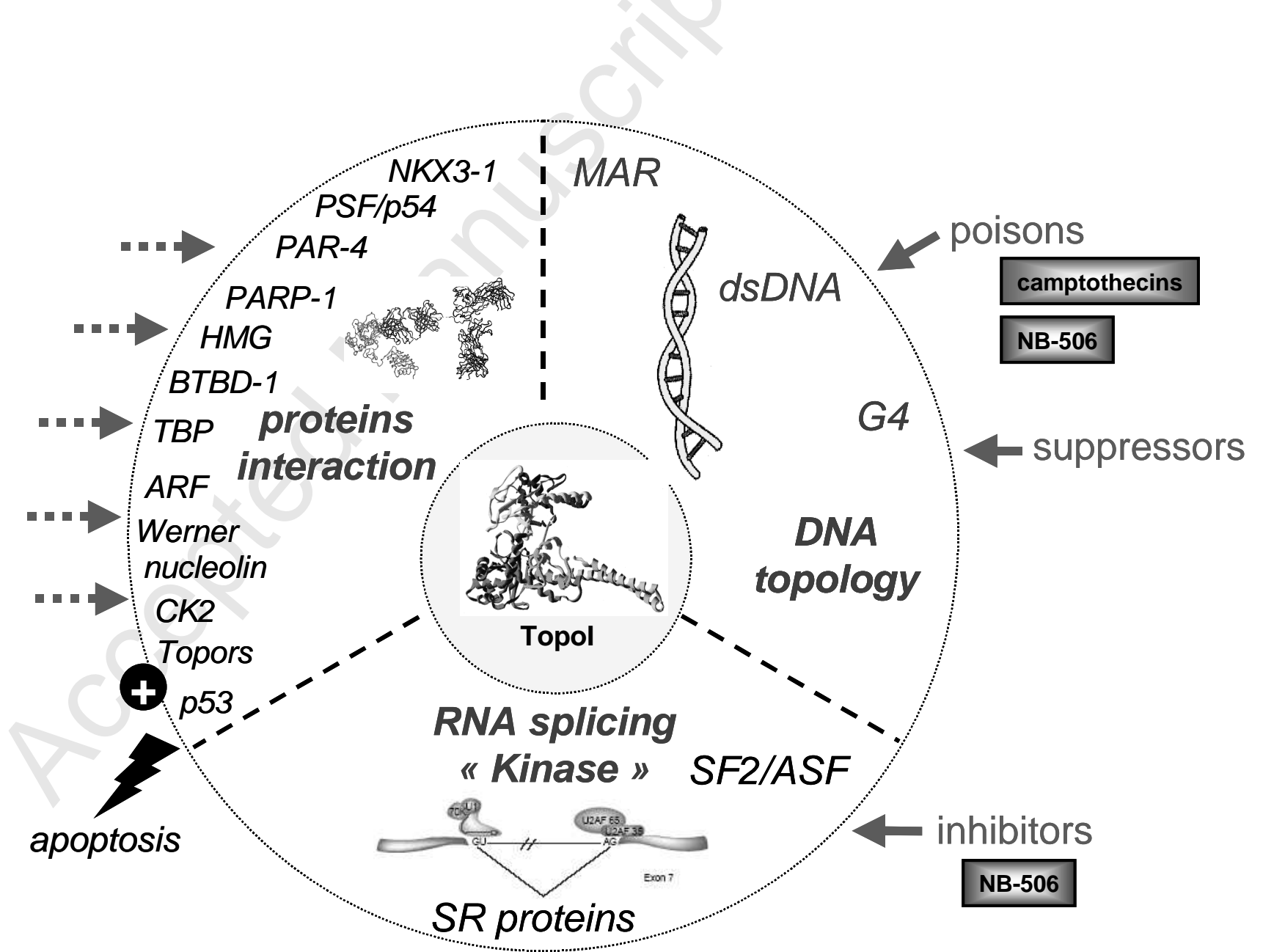


Figure 4

C. Bailly, BCP 10035, graphical abstract

