A Platinum Open Access Journal for Organic Chemistry

Review

Free to Authors and Readers | DOAJ Seal | *Arkivoc* **2022**, part ii, 167-179

Of magic bullets, multiple targets, and artificial intelligence. Are we all set to defeat cancer?

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Received 10-27-2021 **Accepted** 02-24-2022 **Published on line** 03-13-2022

Dedicated to Prof. Girolamo Cirrincione on the occasion of his retirement

Abstract

Personalized medicine applied to patients with complex diseases, like cancer, grants a better quality of life and longer survival. However, in most instances it is not curative, due to the onset of two main obstacles represented by undesired toxicity and/or drug resistance, which contribute to a different extent depending upon the inhibition mechanism. In this short commentary, we try to combine the idea of multi-targeting agents with an Artificial Intelligence platform to further improve patients' treatment and allowing them to be cured or live essentially disease-free.

Keywords: Personalized medicine, anticancer strategies, resistance, toxicity

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1. Introduction

Cancer is a leading cause of death. Worldwide, an estimated 19.3 million new cancer cases and 10.0 million cancer deaths occurred in 2020. The global cancer burden is expected to reach 28.4 million cases in 2040, a 47% rise from 2020, which might get even worse due to increasing risk factors associated with globalization and a growing economy.[1,2] Figures that deserve in-depth reflection and make us feel inadequate, especially if we are scientists committed to contribute to the field of oncologic medicinal chemistry and pharmacology. This does not mean that we can currently do palliative treatments only. In fact, the search for increasingly effective therapeutic regimens has been remarkably successful. We are in the age of personalized medicine,[3,4] in which it is possible to understand the molecular basis of disease and the correlated genomic aspects. This is particularly true in the case of cancer, which is but one (group) of the many genetic diseases produced by inherited or acquired genomic mutations, impairing the control of cellular replication.[5] Since the metastatic progression of the disease requires several concurrent DNA-damage events affecting the signal transduction pathways, the damage pattern is changing from individual to individual. This requires specific treatments which can be rationally assessed in terms of expected outcome and concurrent risks following appropriate genetic studies. An illuminating example is represented by Imatinib (Gleevec), able to target a specific enzyme produced by a chromosomal translocation occurring in Acute Myeloid Leukemia.[6] Therefore, only patients harboring this translocation could benefit of the drug treatment. These types of innovative approaches can make cancer a manageable chronic disease in which partial remissions alternate with relapses, keeping the disease under control. So, the patient does not die with cancer, but lives with cancer.[7] The quality of life has been greatly improved in terms of nutritional, psychological, and physical performance, but we must humbly admit we are at present still unable to cure cancer, with few exceptions.[8]

2. Recently Approved Anticancer Agents

Numerous excellent reviews on general cancer-related subjects pertinent to this commentary are available. We will cite the most recent ones only, without introducing long lists with similar contents or repeating already dissected issues. Going through recent literature, we note that an impressive number of new compounds were approved for cancer treatment by the American regulatory agency, the FDA.[9, 10]

Anticancer drugs approved by the FDA in 2021, reported in Table 1, are all endowed with high specificity.[11] They belong to two types of molecules, namely high- and low-molecular weight compounds.

Table 1. New anticancer drugs approved by FDA in 2021

#	Name (Producer)	Active species	Therapeutic indication	Composition
1.	Tivdak (Seagen)	tisotumab vedotin-tftv	Recurrent/metastatic cervical cancer	monoclonal antibody bound to cytotoxic auristatin
2.	Exkivity (Takeda)V	mobocertinib	Advanced/metastatic non-small cell lung cancer	EGFR tyrosine kinase inhibitor
3.	Welireg (Merck)	belzutifan	Von Hippel-Lindau disease	HIF-2α transcription factor inhibitor
4.	Truseltiq (Helsinn)	infigratinib	Cholangiocarcinoma	FGFR tyrosine kinase inhibitor
5.	Lumakras (Amgen)	sotorasib	Types of non-small cell lung cancer	kRAS G protein inhibitor
6.	Rybrevant (Jannsen)	amivantamab-vmjw	Subset of non-small cell lung cancer	Bispecific (EGFR/cMet) monoclonal antibody
7.	Zynlonta (ADC Ther)	loncastuximab tesirine-lpyl	Relapsed/refractory large B-cell lymphoma	monoclonal antibody bound to cytotoxic pyrrolobenzodiazepine
8.	Jemperli (GSK)	dostarlimab-gxly	Endometrial cancer	PD-1 monoclonal antibody
9.	Fotivda (AVEO)	tivozanib	Renal cell carcinoma	VEGFR tyrosine kinase inhibitor
10.	Ukoniq (TG Ther.)	umbralisib	Marginal zone/follicular lymphoma	PI3k kinase inhibitor
11.	Tepmetko (EMD Serono)	tepotinib	Non-small cell lung cancer	MET tyrosine kinase inhibitor

2.1. High molecular weight compounds

These drugs are targeted at cancer cell specific components, hence able to kill sick cells while preserving healthy cells. The first group comprises immunotherapy applications of monoclonal antibodies as drugs[12], see entry **8** in Table 1, for treatment of endometrial cancer. Bispecific antibodies represent a more sophisticated evolution recognizing two different antigens (entry **6** in Table 1) specific for two known mutations of EGFR (Epidermal Growth Factor Receptor) and cMET gene in lung cancer. In other applications, antibodies behave as drug carriers

(entries **1** and **7** in Table 1), devised for precisely delivering toxic moieties, linked to them, in solid and liquid tumors.[13]

Novel targets being presently exploited by immunotherapy are checkpoint inhibitors largely employed for melanoma and lung cancer treatment. These antibodies can recognize and bind to protein targets involved in signal transduction processes blocking protein-protein interactions.[14] Examples of this strategy are reported in Table 2. The above inhibitors are particularly effective against melanoma and lung cancer and target checkpoint proteins like PD-1, PD-L1 and CTLA-4. Finally, cancer vaccines[15] like Sipuleucel-T (Provenge, Dendreon) and Salimogene laherparepvec (T-VEC, Amgen) have been developed, which stimulate cytokine production, turning on the cellular immune response against melanoma and prostate cancer. The important advances made by immunotherapy practice in the last few years are verified also when treating metastatic cancers.[16] For example, Pembrolizumab (Table 2) prolonged progression-free survival and life expectancy in patients with locally advanced or metastatic non-small cell lung cancer.[17] As a consequence of high selectivity, immunotherapy shows modest but serious off-target effects,[18] related to interference with the immune system (autoimmunity). Moreover, biological macromolecules suffer limitations[19] which reduce their applicability. First, tumors, which represent most of the real cases awaiting treatment. In addition, like for all protein-based drugs, concerns arise on the preparation and administration modalities, storage, and stability of the pharmaceutical formulation.[20] Finally, bio drugs are remarkably expensive, for example, a single dose of Jemper (500 mg, Table1) costs about 10,000€.

Table 2. Checkpoint inhibitors for cancer immunotherapy

#	Name (producer)	Active species	Therapeutic indication	Target[a]
1	Keytruda (Merck)	penbrolizumab	Melanoma, urinary tract cancer	PD-1
2	Yervoy (BMS)	ipilimumab	Renal carcinoma	CTLA-4
3	Opdivo (BMS)	nivolumab	Renal, Head and Neck cancer	CTLA-4
4	Tecentriq (Genentech)	atezolizumab	Urothelial carcinoma, lung cancer	PD-1L

[a]PD-1, Programmed Cell Death-1; PD-1L, PD-1 Ligand; CTLA-4, Cytotoxic T-Lymphocyte Antigen- 4

2.2. Low molecular weight compounds

The second approach for modern cancer drug discovery is based upon specific and effective recognition by low molecular weight compounds of aberrant/overexpressed proteins causing loss of cell growth control. Many of them are TKIs, as they impair protein phosphorylation processes in cancer cells.[21] A list of such compounds is reported in Figure 1. The second class of synthetic anticancer agents consists of epigenetic inhibitors (Figure 2).[22] Such inhibitors are aimed at chromatin-modifying enzymes such as Methyltransferases and Histone deacetylases. Their role is to make chromatin DNA available for transcription and replication. Processing must be accurately performed to grant physiological balance of growth activation and repression. Imbalance of signalling, with uncontrolled growth, turns healthy cell into cancer cells without changing DNA sequence.[22]

Given the limited specificity of the present inhibitors, it is suggested to perform treatments in combination with checkpoint inhibitors.

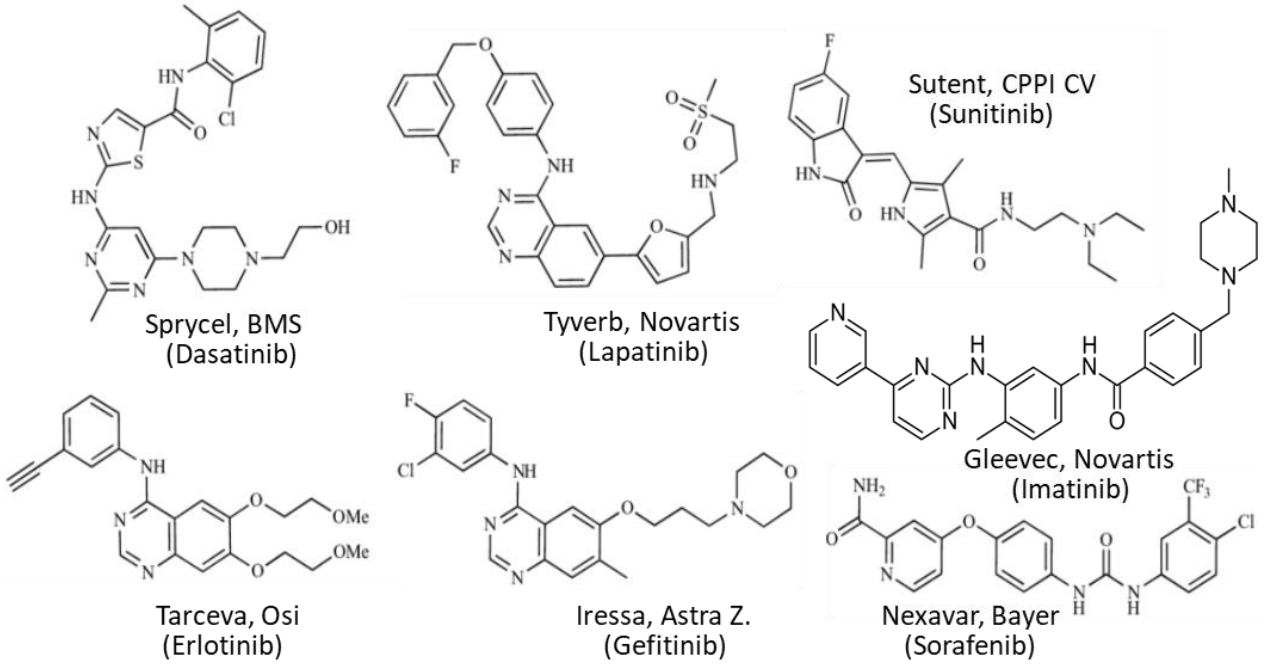

Figure 1. Chemical structure of selected FDA-approved anticancer Tyrosine kinase inhibitors (TKIs).

*** Approved in China**

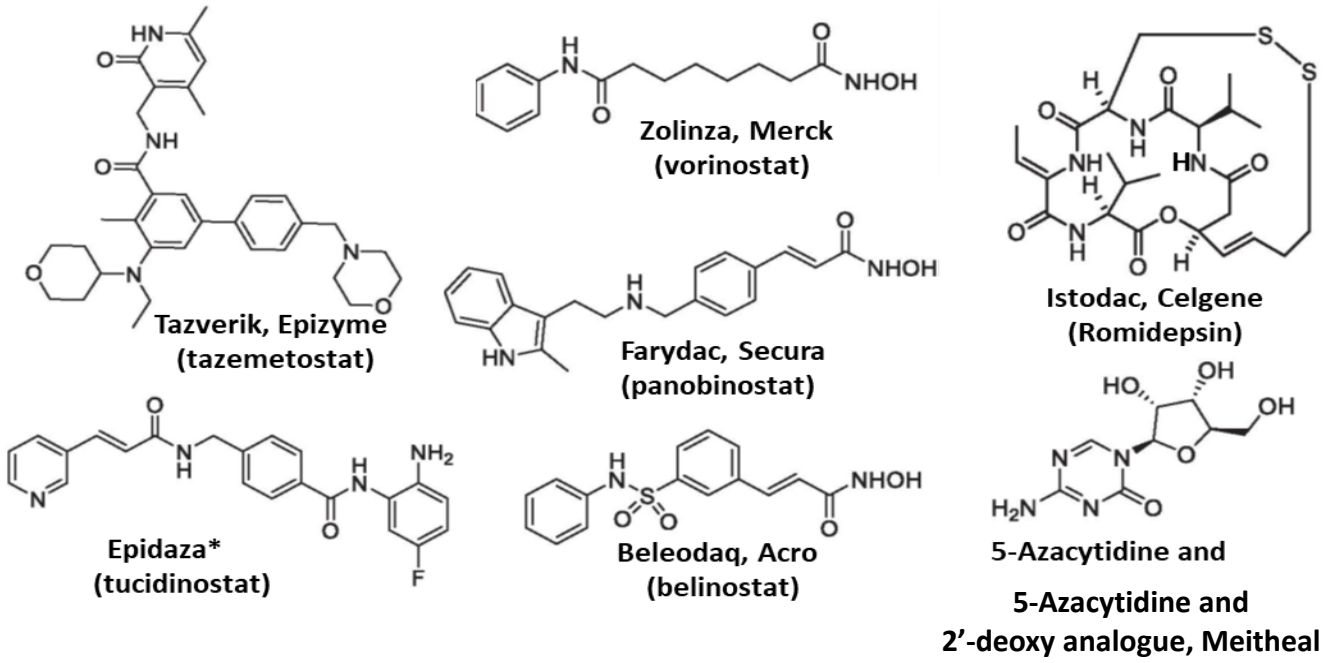

Figure 2. Chemical structure of FDA-approved anticancer epigenetic inhibitors.

From a chemical viewpoint, the small molecule approach is most appealing as it allows the rational synthesis of essentially any new chemical entity, introducing appropriate substituents to optimize target recognition and better address druggability by adjusting ADMET parameters.[23] The history of Gleevec (Figure 1) is a successful example of stepwise modification of an inactive scaffold to obtain selective Tyrosine Kinase activity, with subsequent optimization of hydrophobicity to grant adequate pharmacokinetics.

3. Major Advances in Science and Technologies

In recent times we have also seen spectacular advances in science and technology, especially by the introduction of new research fields fostered by the rapid, substantial improvements in computer science and bioinformatics.[24] In addition, advanced biophysical and biochemical techniques, like Next Generation Sequencing methods, single molecule studies, and atom-level structural determinations of biological macromolecules (cryo Electron Microscopy)[25] have been rendered available to the medicinal chemist as advanced investigation tools, giving invaluable help to deeply understand the molecular events producing (desired and undesired) pharmacological responses, with the final goal to correctly foresee the performance of a newly designed scaffold on a putative receptor before performing synthetic and clinical steps.

4. Important Limitations

Notably, despite the widespread use of the new methods, to date we are still unable to fully describe the pharmacokinetic and pharmacodynamic processes that make the drug act on an identified target. In fact, a non-exhaustive list of the necessary detailed information includes:

- 3D structural properties, acid-base behavior, solubility, tautomeric equilibria, aggregation, H-bonding, permeability, reactivity, and metabolism of isolated drugs.
- 3D and chemical-physical properties of the identified cell target(s).
- *In vivo* kinetic, thermodynamic and structural parameters of the interactive process(es) causing the therapeutic effect, including account of all potential off-target interaction(s). This is not simply related to the principal players, but also to apparently unrelated cell components. For example, a simple bivalent ion can function as a bridge between two side chains of aspartic acid that would otherwise exert a mutual repulsion in the ionized form. Again, divalent metal ions like Mg^{2+} participate in the catalytic activity of DNA processing enzymes, like topoisomerases, by coordinating phosphate oxygens and reducing O-O repulsion in the transition state.[26] As another example, cell membranes represent an additional phase interacting with lipophilic proteins and changing their conformation and distribution.[27]

5. Thermodynamic Considerations

A simple calculation using the thermodynamic correlation between free energy variation, ΔG, and equilibrium binding constant, K (R, universal gas constant, T, absolute temperature):

$$\Delta G = - RT \ln K$$

tells us that for a 100-fold change in affinity at 37 °C the energy into play is about 2.8 Kcal/mol, hardly corresponding to a single hydrogen bond. This means that minor modifications in drug structure may result in

huge changes in affinity and, consequently, in pharmacological response. On the other hand, single mutations in the receptor can fully justify drug resistance, as it occurs in topoisomerase I mutant Gly 503 to Ser, insensitive to poisoning by the chemotherapeutic drug Camptothecin (Sigma).[28] Substantial affinity changes may be not necessarily due to modifications in the binding partners, but also to trivial changes in the environmental setting of living systems. In line with this, different tissues belonging to the same organism can fix different amounts of drug thus generating tissue tropism with uneven distribution of the therapeutic agent.[29]

6. Target Conformation

Another fundamental problem is related to an insufficient level of structural information for quantitatively describing the conformational state of the target macromolecule. In fact, the number of structures (for simplicity let us consider a protein target) available in the Protein Data Bank is about 170,000, which, compared to the 200,000,000 sequences thus far reported[30] represents about 0.1% of the total. It is like solving a 1,000-tile puzzle having just one tile at hand, a difficult job, indeed. In fact, the level of predictability of a target structure starting from the amino acid sequence was until recently unsatisfactory and *in silico* molecular parameters could be at variance compared to the "true" 3D structure obtained from experimental studies. Not having a correct target structure makes drug design poorly significant or, even worse, misleading. Very recently, however, a breakthrough occurred in the field, since Artificial Intelligence deep mind techniques have now been applied to protein structure determinations.[31-33]

7. Artificial Intelligence

Artificial intelligence (AI) is an emerging technology-based platform using sophisticated innovative computational tools like neural networks to effectively mimic human intelligence. AI uses software and systems able to understand and learn from the input data to make independent decisions for achieving specific goals.[34] AI has proved useful in the field of drug discovery and development with an efficiency comparable to other methods.[35] However, a recent application using novel AI deep-mind algorithm (AlphaFold 2), has been outstandingly successful (almost 100% score) in predicting the correct folding of several proteins from sequence data only.[31,32] The method simulates protein structures by considering the whole polypeptide chain, rather than protein domains, folding per se independently of the sequence context of the flanking residues. Hence, prediction allows the consideration of novel, previously unmet types of folding, contextual information from the rest of the sequence and inter-domain packaging. In principle, these innovative approaches allow to safely foresee the structural features of any polypeptide chain, even before synthesizing it, which stands for an immense step forward to the rational design of new effective medicines. There is a corollary to this, since assembly of several protein units often generates targets with superstructural arrangements. This issue has also been examined with AlphaFold in combination with AI program RoseTTAfold.[33] The real performance of AI in rational drug design will be soon assessed by comparing *in silico* predictions with experimental results.

8. Resistance and Toxicity

From the above discussion we can conclude that, although the task remains greatly challenging, we have the analytical-computational tools and the pharmacological chemical-biological knowledge necessary to successfully design new chemical entities as drug candidates, along with an educated forecast of their dynamic and kinetic properties. So far, the answer to the title question, "Are we all set to defeat cancer?", should be: "yes, we are". This seems to be the correct answer even at the beginning of the personalized treatment (see the case of Gleevec, the first selective TKI in clinical practice, Figure 1).[6] Shortly afterwards, however, the drug's potency steadily dropped to levels incompatible with useful treatment, and resistance set on.[34] Irrespective of the drug(s) used, this is a generally occurring event in cancer (and other disease) therapy. If it is difficult to predict the effects of drug-receptor interaction, much more complex will be describing resistance, a multi-factorial process with a concomitant set of different mechanisms, including those of pleiotropic nature, irrespective of the drug's chemical structure with an evolution varying from patient to patient.

Another unavoidable effect of drug administration is toxicity.[35] In general, it is the result of undesired multiple targeting by a drug (or drug metabolite), which effectively binds to cellular components other than the intended receptor. The substantial advances made by pharmacogenomics allow us to identify, genome/transcriptome wide, which genes are affected (under- or over-expressed) in the presence of the drug. Besides gaining valuable information on the intended target(s), we can also highlight and quantify drug driven off-target events. This has two major implications, namely, i) an understanding of the molecular mechanisms of toxicity and their contribution to global drug response. and ii) the potential of exploiting the newly identified binding sites as target(s) for potential drug repurposing.[36] It should however be considered that the on- and off-target effects change from individual to individual depending upon genetic heritage, age, body weight and epigenetic factors. Again, a hardly predictable issue. How could we manage to overcome two tough obstacles such as resistance and toxicity to finally cure cancer?

8.1. Magic bullets

At first sight, the "simple" idea of a drug molecule behaving as a "magic bullet", able to recognize a single known disease-related target specifically and selectively, appears to stand for the most rational approach, and indeed, in many cases efficient drug design has allowed the production of conceptually new important chemical entities. These include the first generation of single-target TKIs, the paradigmatic representative being Gleevec, presently in clinical practice for the treatment of Chronic Myeloid Leukaemia (Figure 1). This therapeutic shows impressive antiproliferative performance at the beginning of therapy. Unfortunately, however, the more selective the drug's binding to the receptor, the faster the onset of resistance, [6] which prevents full eradication of cancer disease. Using a targeted drug mixture produces a better treatment profile but presents a series of further problems related to less predictable pharmacokinetics, heterogeneity of drug mixtures and different administration schedules.[37]

8.2. Multiple targets (polypharmacology)

Hence, the idea of a single molecule shaped to work on several disease-related targets.[37] In fact, this approach was used to design the above mentioned last generation of TKIs, like Sunitinib and Sorafenib (see Figure 1).[38] Besides cancer, this novel line of rational drug design could prove particularly useful in other complex illnesses, involving different biochemical pathways, such as neurological and psychiatric conditions.[39] Here, we should consider that the broadening of target is expected to reduce the occurrence of resistance, especially when several independent signal transduction pathways are simultaneously affected. However, this might

contextually increase the probability of undesired off-target events. This is in fact the case for the second generation TKIs, which recognize at least five different disease-related TKs. On target, they perform better than the parent single-target derivatives; but at the same time as mentioned above, they show cardiac toxicity,[40] unprecedented in the TKI family, due to more extensive off-target binding.

9. Conclusions

Considering the potent and dependable tools we have at hand, we envisage a protocol for the discovery of new (small molecule) multi-targeted anticancer agents, starting from available AI-implemented drug design software[41] to perform the following steps:

- Locate disease-related biochemical pathways.
- Find suitable (protein) targets for efficient pathways inhibition.
- Identify (all) other members of the family to participate in the multitarget process.
- Identify similar, functionally unrelated, proteins present in healthy cells.
- Use AI-driven platforms to safely predict structural features of identified proteins and of the drug-target(s) complex(es).
- Search for structure similarities and differences in the genome-wide collection of potential on- and off-target binding species.
- Exploit similarities to design effective multiple binders to the on-target protein subset.
- Exploit differences to hamper/reduce undesired effects caused by drug binding to the off-target proteins.
- Trim drug structure to a minimum size compatible with efficient inhibition of cancer cells growth.
- Optimize the structure for pharmacokinetics using AI based simulation of ADME parameters, including toxicity.
- Formulate a convenient synthesis of the (few) most promising hits.
- Perform biological and pharmacological testing to validate the multi-target strategy.

According to this procedure, we should rationally (and successfully) devise multitarget low molecular weight agents, acting on different cancer-related biochemical pathways (for instance signal transduction as exemplified by TKIs),[21] yet showing minimal off-target(s) affinity. Clearly, this is not the end of a story, but the beginning of a new scientific adventure based upon more effective and reliable information tools. In fact, we must learn which target inhibitory combinations will produce maximal response and how to be sure we are considering all possible relevant (on-target and off-target) interactions within a cancer (and healthy) cell system. In addition, we should be able to perfect algorithms to describe macromolecular crowding appropriately and accurately,[42] and explain drug-binding to target protein(s) (and protein-nucleic acid assemblies), including *in vivo* induced fit analysis.[43] Finally, even more importantly, we should sensibly improve the available tools to predict resistance and toxicity effects during treatment with a given NCE. Also, new appropriate biomarkers need to be discovered and tuned for personalized applications.

Although we must be cautious and humble in facing up to an ambitious, challenging goal, we believe, with the aid of advanced personalized approaches and AI applications, we are remarkably close to defeat cancer. However, even in the absence of curative magic bullets or gene therapy, today's cancer patients can be treated to live essentially disease-free, with their state of remission constantly monitored. As soon as signs of relapse are recorded, the current treatment must be discontinued and rationally replaced by another personalized

therapy, possibly exploiting different molecular targets. Hence, the continuous need for effective new drugs. In fact, we must always be one step ahead of the disease to manage a satisfactory outcome.

A final remark. We are deeply grateful to Gilmo, to whom this special issue is dedicated, for having put into practice with the commitment of a lifetime the idea that a fruitful collaboration cannot be based on professional grounds only but must be enriched and powered by adding a touch of human warmth and friendship to the qualities of the associated scientists.

References

1. Sun, H.; Ferlay, J.; Siegel, R. L.; Mathieu, L.; Soerjomataram, I. *Ca-Cancer J. Clin.* **2020,** 71, 209-249. https://doi.org/10.3322/caac.21660.
2. Cao, W.; Chen, H.D.; Yu, Y.-W.; Li, N.; Chen, W.-Q.; Jemal, A.; Bray, F. *Chin. Med. J.* **2021**, 134, 783–791. https://doi.org/10.1097/CM9.0000000000001474.
3. Myers, S.H.; Ortega, J.A.; Cavalli, A. *J. Med. Chem*. **2020,** 63, 14151-14183. https://doi.org/10.1021/acs.jmedchem.0c00766
4. Waldman, A.; Fritz, J.M.; Michael, M.; Lenardo, M.J. *Nat. Rev. Immunol.* **2020,** 20, 651-668. https://doi.org/10.1038/s41577-020-0306-5
5. Mardis, E.R. *Curr. Opin. Genet. Dev*. **2012**, 22, 245-250. https://doi.org/10.1016/j.gde.2012.03.005
6. Reichardt, P. *Oncol. Res. Treat*. **2018,** 41, 472-477. https://doi.org/10.1159/000487511
7. Afrasiabi, K.; Linskey, M.E.; Zhou, Y.-H. *Cancers* **2020**, 12, 1649. https://doi.org/10.3390/cancers12061649
8. Vallilas, C.; Sarantis, P.; Kyriazoglou, A.; Koustas, E.; Theocharis, S.; Papavassiliou, A.G.; Karamouzis, M.V. *Int. J. Mol. Sci***. 2021**, 22, 493. https://doi.org/10.3390/ijms22020493
9. Pantzia, P.; Capistrano, R.; De Potter, A.; Vandeborne, L.; Bouche, G. *Front. Pharmacol*. **2021**, 12, 627574. https://doi.org/10.3389/fphar.2021.627574.
10. Bhutani, P.; Gaurav, J.; Raja, N.; Bachhav, N.; Prabhakar, K., Rajanna H. B.; Atish, T. P.; Kumar, R. *J. Med. Chem.* **2020,** 64, 2339-2381. https://doi.org/10.1021/acs.jmedchem.0c01786
11. Drug Approvals and Databases https://www.fda.gov
12. Jain, K.K. *Med. Princ. Pract***. 2021**, 30, 1-16. https://doi.org/10.1159/000511107
13. Dean, A.Q.; Luo, S.; Twomey, J.D.; Zhang, B. *MAbs.* **2021**, 13, 1951427. Correction in: *MAbs***, 2021**, 13, 1966993. https://doi.org/10.1080/19420862.2021.1951427
14. Nakajima, H. T. *Immunol. Med*. **2021**, 4, 10-15. https://doi.org/10.1080/25785826.2020.1785654
15. Blass, E.; Ott, P.A. *Nat. Rev. Clin. Oncol*. **2021**, 18, 215-229.

https://doi.org/10.1038/s41571-020-00460-2
16. Ganesh, K.; Massagué, J. *Nat. Med*. **2021**, 27, 34-44. https://doi.org/10.1038/s41591-020-01195-4
17. Joshi, S.S.; Maron, S.B.; Catenacci, D.V. *Future Oncol***. 2018**, 14, 417-430. https://doi.org/0.2217/fon-2017-0436.
18. Kennedy, L.B.; Salama, A.K.S. *Cancer J. Clin*. **2020**, 70, 86-104. https://doi.org/10.3322/caac.21596
19. Cruz, E.; Kayser, V. *Biologics.* **2019**, 13, 33-51. https://doi.org/10.2147/BTT.S166310
20. Vázquez-Rey, M.; Lang, D.A. *Biotechnol Bioeng*. **2011**, 108, 1494-1508. https://doi.org/0.1002/bit.23155
21. Cicenas, J.; Račien, A. *Cancers* **2021**, 13, 947. https://doi.org/0.3390/cancers13050947
22. Xiao, W.; Zhou, Q.; Wen, X.; Wang, R.; Liu, R.; Wan, T.; Shi, J.; Hu, Y.; Hou, J. *Front. Pharmacol*. **2021**, 12, 702360. https://doi.org/10.3389/fphar.2021.702360
23. Zhong, S.S.; Li, Y.; Xiong, L.; Wang, W.; Wu, M.; Yuan, T.; Yang, W.; Tian, C.; Miao, Z.; Wang, T.; Yang, S. *Signal Transduction and Targeted Ther.* **2021,** 6, 201-248. https://doi.org/10.1038/s41392-021-00572-w
24. MacEachern, S.J.; Forkert, N.D. *Genome*. **2021**, 64, 416-425. https://doi.org/10.1139/gen-2020-0131
25. Cheng, J. *Science.* **2018**, 361, 876-880. https://doi.org/10.1126/science.aat4346
26. Sissi, C.; Palumbo, M. *Nucleic Acids Res.* **2009,** 37, 702-711. https://doi.org/10.1093/nar/gkp024
27. Bolla, J.R.; Agasid, M.T.; Mehmood, S.; Robinson, C.V. *Annu. Rev. Biochem*. **2019**, 88, 85-111. https://doi.org/10.1146/annurev-biochem-013118-111508
28. Tanizawa, A.; Beitrand, R.; Kohlhagen, G; Tabuchi, A.; Jenkins, J, Pommier Y. *J. Biol. Chem*. **1993**, 268, 25463-25468
29. Dubois, M.E.; Yoshihara, P.; Slifka, M.K. *Semin. Respir. Crit. Care Med*. **2005**, 26, 635-642. https://doi.org/10.1055/s-2005-925527
30. Berman, H.M.; Vallat, B.; Lawson, C.L. *IUCrJ.* **2020**, 7, 630-638. https://doi.org/10.1107/S205225252000562X.
31. Jumper, J.; Evans, R.; Pritzel, A.; Green, T.; Figurnov, M.; Ronneberger, O.; Tunyasuvunakool, K.; Bates, R.; Žídek, A.; Potapenko, A.; Bridgland, A.; Meyer, C.; S Kohl, S.A.A.; Ballard, A.J.; Cowie, A.; Romera-Paredes, B.; Nikolov, S.; Jain, R.; Adler, J.; Back, T.; Petersen, S.; Reiman, D.; Clancy, E.; Steinegger, M.; Pacholska , M.; Berghammer, T.; Silver, D.; Vinyals, O.; Senior, A.W.; Kavukcuoglu, K.; Kohli, P. Hassabis, D. *Nature.* **2021**, 596, 583-589. https://doi.org/10.1038/s41586-021-03819-2.
32. Tunyasuvunakool, K.; Adler, J.; Wu, Z., Green, T., Zielinski, M.; Žídek, A.; Bridgland, A.; Cowie, A.; Meyer, C.; Laydon, A.; Velankar, S.; Kleywegt, G.; Bateman, A.; Evans, R.; Pritzel, A.; Figurnov, M.; Ronneberger, O.; Bates, R.; Kohl, S.A.A.; Potapenko, A.; Ballard, A.J.; Romera-Paredes, B.; Nikolov, S.; Jain, R.; Clancy, E.; Reiman, D.; Petersen, S.; Senior, A.W.; Kavukcuoglu, K.; Birney, E.; Kohli, P.; Hassabis, D. *Nature*. **2021**, 596, 590-596. https://doi.org/10.1038/s415828-1

33. Humphreys, R.; Pei, J.; Baek, M.; Krishnakumar, A.; Anishchenko, I.; Ovchinnikov, S.; Zhang, J.; Ness, T.J.; Banjade, S.; Bagde, S.R.; Stancheva, V.G.; Li, X.; Liu, K.; Zheng, Z.; Barrero, D.J.; Roy, U.; Kuper, B.; Branzei, D.; Rizo, J.; Kisker, C.; Greene, E.C.; Biggins, S.; Keeney, S.; Miller, E.A.; Fromme, J.C.; Hendrickson, T.L.; Cong, Q.; Baker, D. *Science.* **2021**, 374, 6573. https://doi.org/10.1126/science.abm480
34. Gottesman, M.M. *Annu. Rev. Med*. **2002**, 53, 615-627. https://doi.org/10.1146/annurev.med.53.082901.103929.
35. Cleeland,C.S.; Allen, J.D.; Roberts, S.A.; Brell, J.M.; Giralt, S.A.; Khakoo, A.Y.; Kirch, R.A.; Kwitkowski, V.E.; Liao, Z.; Skillings, J. *Nat. Rev. Clin. Oncol*. **2012**, 9, 471-478. https://doi.org/10.1038/nrclinonc.2012.99
36. Palve, V.; Liao,Y.; Rix, R.L.L.; Rix, U. *Semin. Cancer Biol*. **2021**, 68, 209-229. https://doi.org/10.1016/j.semcancer.2020.02.003.
37. Petrelli, A.; Giordano, S. *Curr. Med. Chem*. **2008**, 15, 422-432 https://doi.org/10.2174/092986708783503212.
38. Guo, T.; Ma, S. ChemMedChem. **2021,** 16, 600-620. https://doi.org/10.1002/cmdc.202000658.
39. Oña ,G.; Bouso,C. *Curr. Med .Chem.* **2021**, *28*, 53-68. https://doi.org/10.2174/0929867326666191212103330.
40. Lamore, S.D.; Kohnken, R.A.; Peters, M.S.; Kolaja, K.L. *Chem Res Toxicol*. **2020**, 33, 125-136. http://doi.org/10.1021/acs.chemrestox.9b00387.
41. Zhu, H. *Annu. Rev. Pharmacol. Toxicol*. **2020**, 60, 583-589. https://doi.org/10.1146/annurev-pharmtox-010919-023324.
42. Model, M.A.; Hollembeak, J.E.; Kurokawa, M. *Cell Physiol. Biochem*. **2021**, 55, 25-40. https://doi.org/10.33594/000000319.
43. Redhair, M.; Atkins, W.M. *Arch. Biochem. Biophys*. **2021**, 714, 109064. https://doi.org/10.1016/j.abb.2021.109064.

Authors' Biographies

Manlio Palumbo, Ph.D. in Chemistry in 1970, was appointed as full professor of Medicinal Chemistry in 1990. He spent two years abroad (Univ. of California, San Diego, and Max Planck Institut für Biochemie, Munich, Germany)

performing conformational studies on bioactive peptides and poly(amino acids). Subsequently, he focused his research on the mechanisms of action of chemotherapeutic drugs using biophysics and medicinal chemistry techniques to understand the molecular basis for specific drug-DNA recognition, also in the presence of DNA-processing enzymes (topoisomerases and telomerase). He authored more than 250 publications in international journals and presented over 300 communications to congresses, many of which as an invited lecturer. He serves as an official reviewer for more than 15 international journals and is/was in the Editorial Advisory Board of four of them. He served as the Editor of the book "Advances in DNA sequence-specific agents", JAI Press, Greenwich, USA and as a Guest Editor in thematic issues. Manlio Palumbo's list of national and international collaborations include numerous outstanding groups in Europe and in the United States. Moreover, he was in the Board of Experts of leading Scientific European Institutions. He headed an active research group since 1980 and received national and international funds to support his work in the medicinal chemistry and biomedicine field. In the period 2001-2005 he served as the Head of the Department of Pharmaceutical Sciences at the University of Padova. From 2004 to 2008 he was President of the Interfaculty Course in Health Biotechnologies. From 2005 to 2009 he was appointed as Vice-rector at the University of Padova. From 2012 to 2015he served as the Head of the new Department of Pharmaceutical and Pharmacological Sciences, University of Padova. In 2015 he was the recipient of the Giacomello Medal award of the Italian Chemical Society for his research achievements in the field of Medicinal Chemistry. As mandatory by law, he retired in 2017 and is now associated to the University of Padova as a Senior Research Scientist.

Prof. Claudia Sissi received her degree in Medicinal Chemistry and Technology in 1991 and the PhD degree in Medicinal Chemistry in 1994 from Padova University, Italy. After post-doc positions at Yale University, New Haven CT, USA; Istituto Italiano per lo Studio e la Cura dei Tumori, Milan, Italy; Glaxo, Verona, Italy, in 2004 she was appointed at the Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Italy, as Research Associate, in 2009 as Associate Professor and in 2015 as Full Professor. She held courses in Medicinal Chemistry, Pharmaceutical Biotechnology and Biopharmaceutical Analysis. Currently she is vice-coordinator of the PhD course in Molecular Sciences and member of CRIBI (Research Center for Innovative Biotechnologies). Her main research interests cover: 1) Conformational equilibria of nucleic acids, 2) Molecular mechanism of drug-macromolecule recognition & 3) Molecular Basis of ant topoisomerase and antithrombotic drugs. She holds active international collaborations with several groups recognized for their outstanding research in the above fields. She authored 130 publications in international journals, 3 books, 2 patents and presented over 50 oral communications at international congresses as invited lecturer. She is in the Editorial Advisory Board of several international journals, and she is a member of AACR, ACS and SCI.

