

DEBATE

Open Access



In silico cancer research towards 3R

Claire Jean-Quartier¹, Fleur Jeanquartier^{1,2*} , Igor Jurisica³ and Andreas Holzinger^{1,2}

Abstract

Background: Improving our understanding of cancer and other complex diseases requires integrating diverse data sets and algorithms. Intertwining *in vivo* and *in vitro* data and *in silico* models are paramount to overcome intrinsic difficulties given by data complexity. Importantly, this approach also helps to uncover underlying molecular mechanisms. Over the years, research has introduced multiple biochemical and computational methods to study the disease, many of which require animal experiments. However, modeling systems and the comparison of cellular processes in both eukaryotes and prokaryotes help to understand specific aspects of uncontrolled cell growth, eventually leading to improved planning of future experiments. According to the principles for humane techniques milestones in alternative animal testing involve *in vitro* methods such as cell-based models and microfluidic chips, as well as clinical tests of microdosing and imaging. Up-to-date, the range of alternative methods has expanded towards computational approaches, based on the use of information from past *in vitro* and *in vivo* experiments. In fact, *in silico* techniques are often underrated but can be vital to understanding fundamental processes in cancer. They can rival accuracy of biological assays, and they can provide essential focus and direction to reduce experimental cost.

Main body: We give an overview on *in vivo*, *in vitro* and *in silico* methods used in cancer research. Common models as cell-lines, xenografts, or genetically modified rodents reflect relevant pathological processes to a different degree, but can not replicate the full spectrum of human disease. There is an increasing importance of computational biology, advancing from the task of assisting biological analysis with network biology approaches as the basis for understanding a cell's functional organization up to model building for predictive systems.

Conclusion: Underlining and extending the *in silico* approach with respect to the **3Rs for replacement, reduction and refinement** will lead cancer research towards *efficient* and *effective* precision medicine. Therefore, we suggest refined translational models and testing methods based on integrative analyses and the incorporation of computational biology within cancer research.

Keywords: Cancer research, Computational biology, Cancer bioinformatics, Integrative analysis, *In silico* modeling, *In vitro* methods, *In vivo* techniques, *Ex vivo* systems, Tumor growth, Alternative animal experimentation, 3Rs

Background

Cancer remains to be one of the top causes of disease-related death. World Health Organization (WHO) reported 8.8 million cancer-related deaths in 2015 [1]. Around one out of 250 people will develop cancer each year, and every fourth will die from it [2]. WHO estimates the number of new cases will rise by ~70% over the next twenty years. Despite decades of research [3], mortality rates and recurrence remain high, and we have limited

options for effective therapies or strategies regarding cancer prevention.

Tumor cells exhibit chaotic, heterogeneous and highly differentiated structures, which is determinative to the lack of effective anticancer drugs [4]. For that matter, predictive preclinical models that integrate *in vivo*, *in vitro* and *in silico* experiments, are rare but necessary for the process of understanding tumor complexity.

A biological system comprises a multiplicity of interconnected dynamic processes at different time and spatial range. The complexity often hinders the ability to detail relationships between cause and effect. Model-based approaches help to interpret complex and variable structures of a system and can account for biological mechanisms. Next to studying pathological processes or

*Correspondence: fleur.jeanquartier@tugraz.at

¹Holzinger Group, Institute for Medical Informatics, Statistics and Documentation, Medical University Graz, Graz, Austria

²Institute of Interactive Systems and Data Science, Graz University of Technology, Graz, Austria

Full list of author information is available at the end of the article

molecular mechanisms, they can be used for biomarker discovery, validation, basic approaches to therapy and preclinical testing. So far, preclinical research primarily involves *in vivo* models based on animal experimentation.

Intertwining biological experiments with computational analyses and modeling may help to reduce the number of experiments required, and improve the quality of information gained from them [5]. Instead of broad high-throughput screens, focused screens can lead to increased sensitivity, improved validation rates, and reduced requirements for *in vitro* and *in vivo* experiments. For Austria, the estimated number of laboratory animal kills per year was over 200 000 [6]. In Germany the number of animal experiments for research is estimated as 2.8 millions [7]. Worldwide, the quantity of killed animals for research, teaching, testing and experimentation exceeds 100 000 000 per year [6–14], as shown in Fig. 1.

Principles for humane techniques were classified as replacement, reduction and refinement, also known as the 3Rs [15]. While most countries follow recommendations of Research Ethics Boards [16], discussion of ethical issues regarding the use of animals in research continues [17]. So far, 3R principles have been integrated into legislation and guidelines how to execute experiments using animal models, still, rethinking of refined experimentation will ultimately lead to higher-quality science [18]. The 3R concept also implies economic, ethical and academic sense behind sharing experimental animal resources, making biomedical research data scientifically easily available [19]. The idea behind 3R has been implemented in several programs such as Tox21 and ToxCast also offering high throughput assay screening data on several cancer-causing compounds for bioactivity profiles and predictive models [20–22].

It is clear that no model is perfect, and is lacking some aspects of reality. Thus, one has to choose and use appropriate models to advance specific experiments. Cancer research relies on diverse data from clinical trials, *in vivo* screens and validation studies, and functional studies

using diverse *in vitro* experimental methods, such as cell-based models, spheroid systems, and screening systems for cytotoxicity, mutagenicity and cancerogenesis [23, 24]. New technologies will advance in organ-on-a-chip technologies [25] but also include the *in silico* branch of systems biology with its goal to create the virtual physiological human [26]. The range of alternative methods has already expanded further towards *in silico* experimentation standing for “performed on a computer”. These computational approaches include storage, exchange and use of information from past *in vitro* and *in vivo* experiments, predictions and modeling techniques [27]. In this regard, the term *non-testing* methods has been introduced, which summarizes the approach in predictive toxicology using previously given information for risk assessment of chemicals [28]. Such methods generate *non-testing* data by the general approach of grouping, (quantitative) structure-activity relationships (QSAR) or comprehensive expert systems, which are respectively based on the similarity principle [29–31].

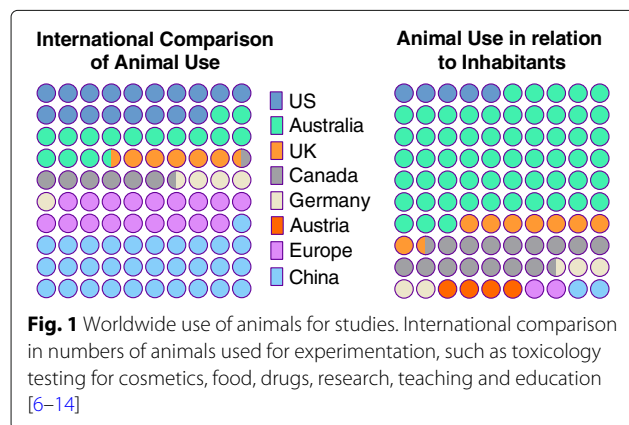
The regulation of the European Union for registration, evaluation, authorisation and restriction of chemicals (REACH) promotes adaptation of *in vivo* experimentation under the conditions that *non-testing methods* or *in vitro* methods provide valid, reliable, relevant information, adequate for the intended purpose, or in case that testing is technically impossible [30].

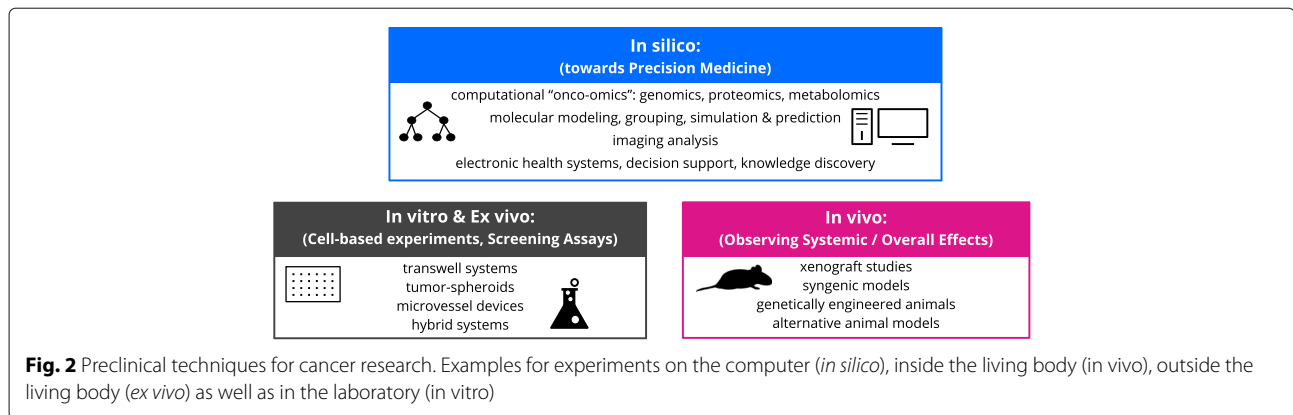
Generally, *in vitro* and *in silico* are useful resources for predicting several (bio)chemical and (patho)physiological characteristics of likewise potential drugs or toxic compounds, but have not been fit for full pharmacokinetic profiling yet [32]. *In vitro* as well as *in silico* models abound especially in the fields of toxicology and cosmetics, based on cell culture, tissues and simulations [33]. In terms of 3R, *in vitro* techniques allow to reduce, refine and replace animal experiments. Still, wet biomedical research requires numerous resources from a variety of biological sources. *In silico* methods can further be used to augment and refine *in vivo* and *in vitro* models. Validation of computational models will still require results from *in vivo* and *in vitro* experiments. Though, in the long run, integrative approaches incorporating computational biology will reduce laboratory work in the first place and effectively succeed in 3R.

Within the next sections, we summarize common methods and novel techniques regarding *in vivo*, *in vitro* and *in silico* cancer research, presented as overview in Fig. 2, and associated modeling examples listed in Table 1.

In vivo methods

Animals are the primary resource for research on the pathogenesis of cancer. Animal models are commonly used for studies on cancer biology and genetics as well





as the preclinical investigation of cancer-therapy and the efficacy and safety of novel drugs [34]. Animal models represent the *in vivo* counterpart to cell-lines and suspension culture, while being superior in terms of physiological relevance offering imitation of parental tumors

Table 1 Overview of exemplary models for cancer research

	References
In vivo	
Murine models	[37]
Genetically engineered mouse model	[36]
Zebra fish model	[42]
Drosophila model	[41]
Chick embryo model	[43, 44]
In vitro	
General 2D/3D <i>in vitro</i> models	[46, 53]
Transwell model	[48]
Spheroid system	[49]
Microfluidic system	[50]
Tissue-engineered microvessel model	[51]
In silico	
Sequence analysis	[63, 69, 74]
General pathway analysis and network inference	[132, 133, 135]
Pan-cancer	[62, 82, 134, 139]
Chemical perturbation mapping	[64, 66, 68]
Pharmacogenomic mapping	[99, 102, 117, 136]
Genome-phenotype mapping	[81]
Clinical data integration	[106]
Structure mapping	[102, 103]
Structure and activity	[100, 101]
Framework for key events and mode of action	[97, 98]
Image classification	[85, 87]
Growth prediction	[91–93]

and a heterogeneous microenvironment as part of an interacting complex biochemical system.

In general, animal models primarily based on murine or rodent models can be subdivided into the following groups of (I) xenograft models, which refer to the heterotopic, subcutaneous intraperitoneal or orthotopic implantation into SCID (Severe Combined Immune Deficiency) or nude mice, (II) syngenic models involving the implantation of cells from the same strain into non-immunocompromised mice, and (III) genetically engineered models, which allow for RNA interference, multigenic mutation, inducible or reversible gene expression [35, 36].

Several engineered mouse models on cancer and related diseases have been developed so far [37]. In case of xenograft models, tumor-specific cells are transplanted into immunocompromised mice. Common tumor xenograft models lack the immune system response that can be crucial in tumor development and progression [38]. Xenograft models can be patient-derived, by transference of a patient's primary tumor cells after surgery into immunocompromised mice. The transplantation of immortalized tumor cell-lines represents a simplified preclinical model with limited clinical application possibilities [39]. For these reasons, there is a trend towards genetically engineered animal models, allowing for site-directed mutations on tumor-suppressor genes and proto-oncogenes as the basis for studies on oncogenesis [40].

Next to the gold standard of murine and rodent models, there are other animal model systems frequently used, such as the *Drosophila melanogaster* (fruit fly) or *Danio rerio* (zebra fish) [41, 42]. The fruit fly offers the advantage of low-cost handling and easy mutant generation while it holds a substantially high conservation of the human cancer-related signaling apparatus [41]. There are additional animal models, commonly referred to as alternatives, such as zebra fish models for angiogenesis studies and chick embryo CAM (chorioallantoic

membrane) models, offering rapid tumor formation due to the highly vascularized CAM structure [40, 43, 44].

So far, preclinical model systems do not provide sufficient information on target validation, but aid in identifying and selecting novel targets, while new strategies offer a quantitative translation from preclinical studies to clinical applications [45].

In vitro methods

In vitro models offer possibilities for studying several cellular aspects as the tumor microenvironment using specific cell types, extracellular matrices, and soluble factors [46]. In vitro models are mainly based on either cell cultures of adherent monolayers or free-floating suspension cells [47]. They can be categorized into: (I) transwell-based models which include invasion and migration assays [48], (II) spheroid-based models involving non-adherent surfaces [49], hanging droplets and microfluidic devices [50], (III) tumor-microvessel models which come with predefined ECM (extracellular matrix) scaffolds and microvessel self-assemblies [51], and (IV) hybrid tumor models including embedded *ex vivo* tumor sections, 3D invasion through clusters embedded in gel, and 2D vascular microfluidics [52].

Generally, such cell culture models focus on key aspects of metabolism, absorption, distribution, excretion of chemicals or other aspects of cell signaling pathways, such as aspects of metastasis under a controlled environment [53]. Scale-up systems attempt to emulate the physiological variability in order to extrapolate from in vitro to in vivo [54]. Advanced models as 3D culture systems more accurately represent the tumor environment [55]. Cell culture techniques include the formation of cell spheroids, which are frequently used in cancer research for approximating in vitro tumor growth as well as tumor invasion [56]. In particular, multicellular tumor spheroids have been applied for drug screening and studies on proliferation, migration, invasion, immune interactions, remodeling, angiogenesis and interactions between tumor cells and the microenvironment [46].

In vitro methods include studies on intercellular, intracellular or even intraorganellar processes, which determine the complexity of tumor growth to cancerogenesis and metastasis, based on several methods from the disciplines of biophysics, biochemistry and molecular biology [23].

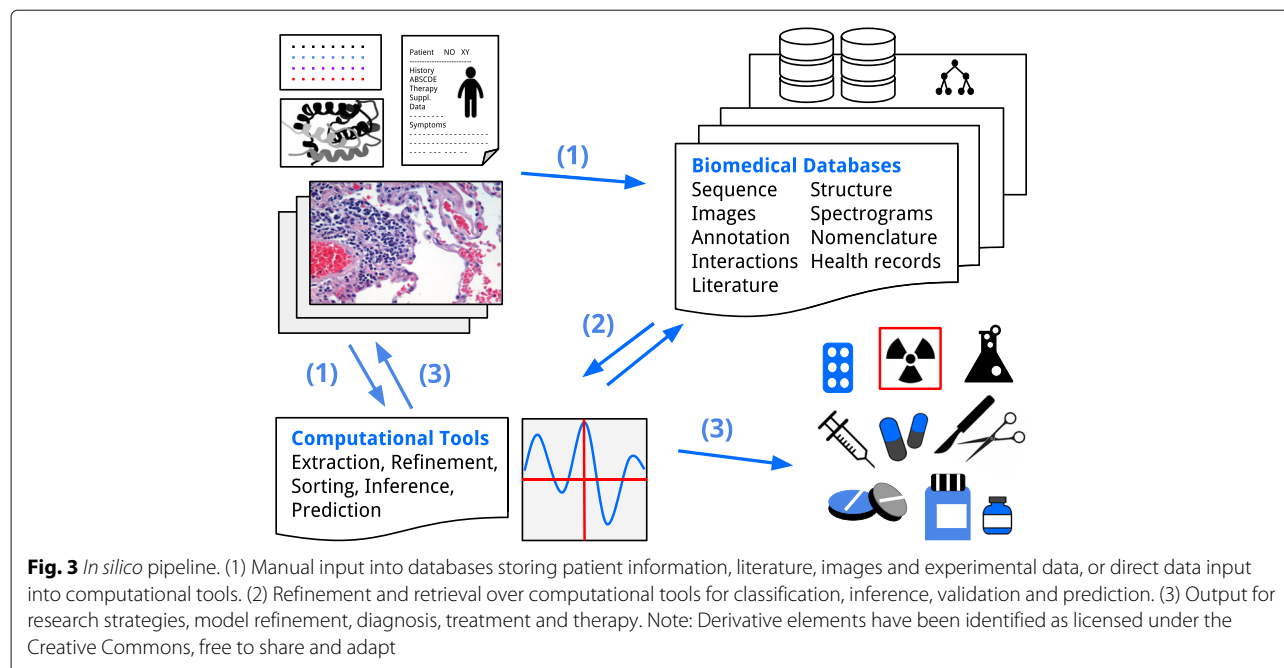
Ex vivo systems offer additional possibilities to study molecular features. Such systems can be derived from animal and human organs or multiple donors. Thereby, *ex vivo* systems comprise the isolation of primary material from an organism, cultivation and storage in vitro and differentiation into different cell types [57]. In this regard, induced pluripotent stem cells, in particular cancer stem cell subpopulations, have been presented as in

vitro alternative to xenograft experiments [58]. Moreover, *ex vivo* methods can be used to predict drug response in cancer patients [59]. These systems have been developed to improve basic in vitro cell cultures while overcoming shortcomings of preclinical animal models; thus, serving as more clinically relevant models [60].

In silico analysis

The term *in silico* was created in line with in vivo and in vitro, and refers to as performed on computer or via computer simulation [28]. *In silico* techniques can be summarized as the process of integrating computational approaches to biological analysis and simulation. So far, *in silico* cancer research involves several techniques including computational validation, classification, inference, prediction, as well as mathematical and computational modeling, summarized in Fig. 3. Computational biology and bioinformatics are mostly used to store and process large-scale experimental data, extract and provide information as well as develop integrative tools to support analysis tasks and to produce biological insights. Existing well-maintained databases provide, integrate and annotate "information on various cancers [61], and are increasingly being used to generate predictive models, which in turn will inform and guide biomedical experiments. Table 2 lists several representative examples of such databases.

The Cancer genome project and Cancer Genome Atlas have generated an abundance of data on molecular alterations related to cancer [62]. The Cancer Genome Anatomy Project by the National Cancer Institute also provides information on healthy and cancer patient gene expression profiles and proteomic data with the objective to generate novel detection, diagnosis and treatment possibilities [63]. In this connection, analyzing molecular changes and collecting gene expression signatures of malignant cells is important for understanding cancer progression. As example, over a million profiles of genes, drugs and disease states have been collected as so-called cellular connectivity maps in order to discover new therapeutic targets for treating cancer [64]. Regarding the effect of small molecules on human health, computational toxicology has created *in silico* resources to organise, analyse, simulate, visualise, or predict toxicity as a measure of adverse effects of chemicals [31, 65]. Large-scale toxicogenomics data has been collected by multi-agency toxicity testing initiatives, for forecasting carcinogenicity or mutagenicity [20, 66–68]. Thereby, gene expression signatures and information on chemical pathway perturbation by carcinogenic and mutagenic compounds have been analyzed and incorporated into *in silico* models to predict the potential of hazard pathway activation including carcinogenicity to humans [20–22, 66].



The analysis of genomic and proteomic data largely focuses on comparison of annotated data sets, by applying diverse machine learning and statistical methods. Most genomic alterations comprise single nucleotide variants, short base insertions or deletions, gene copy number variants and sequence translocations [69]. Thereby, cancer genes are defined by genetic alterations, specifically selected from the cancer microenvironment, conferring an advantage on cancer cell growth. In this regard, the

goal is set in characterizing driver genes. However, combination of such genes may provide prognostic signatures with clear clinical use. Integrating patterns of deregulated genome or proteome with information about biomolecular function and signaling cascades does in turn provide inside into underlying biological mechanism driving the disease.

Analysis of genomic and proteomic data relies on processing methods such as clustering algorithms [70].

Table 2 List of main databases and data resources in cancer research

Name	Url	References
cBioPortal	http://www.cbioportal.org	[116, 128]
BioPreDyn-Bench	https://sites.google.com/site/biopredynbenchmarks/	[105]
CGAP	https://cgap.nci.nih.gov/	[63]
EPA Toxcast Screening Library	https://actor.epa.gov/dashboard/	[21, 66]
EpiFactors	http://epifactors.autosome.ru/	[77]
Human Protein Atlas	https://www.proteinatlas.org/	[111, 112]
GDSC	http://www.cancerrxgene.org/	[102, 104]
GDC/TCGA	https://portal.gdc.cancer.gov/	[62, 139]
Gene Ontology	http://www.geneontology.org/	[129]
KEGG	http://www.genome.jp/kegg/	[131]
NCI-60 databases	https://discover.nci.nih.gov/cellminer/	[116, 145]
Open TG-GATes	http://toxico.nibiohn.go.jp/english/	[67]
Reactome	https://reactome.org/	[130]
pathDIP	http://ophid.utoronto.ca/pathdip/	[135]

Cluster analysis depicts the statistical process of group formation upon similarities, exemplary for exploratory data mining [71]. Understanding the heterogeneity of cancer diseases and the underlying individual variations requires translational personalized research such as statistical inference at the patient level [72]. Statistical inference represents the process of detailed reflections on data and deriving sample distributions, understanding large sample properties and concluding with scientific findings as knowledge discovery and decision making. This computational approach involving mathematical and biological modeling, allows to predict disease risk and progression [72].

Besides directly studying cancer genes and proteins, it is increasingly recognized that their regulators, not only involving so far known tumor suppressor genes and proto-oncogenes but also non-coding elements [73–75] and epigenetic factors in general can be highly altered in cancer [76, 77]. These include metabolic cofactors [78], chemical modifications such as DNA methylation [79], and microRNAs [80]. Another approach to studying cancer involves the view of dysregulated pathways instead of single genetic mutations [81]. The heterogeneous patient profiles are thereby analyzed for pathway similarities in order to define phenotypic subclasses related to genotypic causes to cancer. Next to elucidating novel genetic players in cancer diseases using genomic patient profiling, there are other studies focusing the underlying structural components of interacting protein residues in cancer [82]. This genomic-proteomic-structural approach is used to highlight functionally important genes in cancer. In this regard, studies on macromolecular structure and dynamics give insight into cellular processes as well as dysfunctions [83].

Image analysis and interpretation strongly benefit from diverse computational methods in general and within the field of cancer therapy and research. Computer algorithms are frequently used for classification purposes and assessment of images in order to increase throughput and generate objective results [84–86]. Image analysis via computerized tomography has been recently proposed for evaluating individualized tumor responses [87]. Pattern recognition describes a major example on extracting knowledge from imaging data. Recently, an algorithmic recognition approach of the underlying spatially resolved biochemical composition, within normal and diseased states, has been described for spectroscopic imaging [88]. Such an approach could serve as digital diagnostic resource for identifying cancer conditions, and complementing traditional diagnostic tests towards personalized medicine.

Computational biology provides resources and tools necessary for biologically-meaningful simulations, implementing powerful models of cancer using experimental data, supporting trend analysis, disease progression and

strategic therapy assessment. Network models on cancer signaling have been build on the basis of time-course experiments measuring protein expression and activity in use of validating simulation prediction and testing drug target efficacy [89]. Simulations of metabolic events have been introduced with genome scale metabolic models for data interpretation, flux prediction, hypothesis testing, diagnostics, biomarker and drug target identification [90]. Mathematical and computational modeling have been further used to better understand cancer evolution [91–93].

Since the concept of 3R has its primary focus on replacing animal experimentation within the area of chemical assessment, several *in silico* methods have been or are being developed in the field of toxicology. So far, computational toxicology deals with the assessment of hazardous chemicals such as carcinogens rather than computational biomedicine and biological research concerning cancer. Still, underlying methods can be likewise integrated into both disciplines [94, 95]. Recently, toxicology has brought up the adverse outcome pathway (AOP) methodology, which is intended to collect, organise and evaluate relevant information on biological and toxicological effects of chemicals, more specifically, existing knowledge concerning biologically plausible and empirically supported links between molecular-level perturbation of a biological system and an adverse outcome at the level of biological organisation of regulatory concern [96, 97]. This framework is intended to focus humans as model organism on different biological levels rather than whole-animal models [95]. The International Program on Chemical Safety has also published a framework for analyzing the relevance of a cancer mode of action for humans, formerly assessed for carcinogenesis in animals [98]. The postulated mode of action comprises a description of critical and measurable key events leading to cancer. This framework has been integrated into the guidelines on risk assessment by the Environmental Protection Agency to provide a tool for harmonization and transparency of information on carcinogenic effect on humans, likewise intended to support risk assessors and also the research community. Noteworthy, next to frameworks, there are several common toxicological *in silico* techniques. Especially similarity methods play a fundamental role in computational toxicology with QSAR modeling as the most prominent example [28, 29]. QSARs mathematically relate structure-derived parameters, so-called molecular descriptors, to a measure of property or activity. Thereby, regression analysis and classification methods are used to generate a continuous or categorical result as qualitative or quantitative endpoint [29, 31]. Exemplary, models based on structure and activity data have been used to predict human toxicity endpoints for a number of carcinogens [22, 99–101]. Still, in order to predict drug

efficacy and sensitivity, it is suggested to combine models on chemical features such as structure data with genomic features [102–104].

Combined, *in silico* methods can be used for both characterization and prediction. Thereby, simulations are frequently applied for the systematic analysis of cellular processes. Large-scale models on whole biological systems, including signal-transduction and metabolic pathways, face several challenges of accounted parameters at the cost of computing power [105]. Still, the complexity and heterogeneity of cancer as well as the corresponding vast amount of available data, asks for a systemic approach such as computational modeling and machine learning [106, 107]. Overall, *in silico* biological systems, especially integrated mathematical models, provide significant link and enrichment of *in vitro* and *in vivo* systems [108].

Computational cancer research towards precision medicine

Oncogenesis and tumor progression of each patient are characterized by multitude of genomic perturbation events, resulting in diverse perturbations of signaling cascades, and thus requiring thorough molecular characterization for designing effective targeted therapies [109]. Precision medicine customizes healthcare by optimizing treatment to the individual requirements of a patient, often based on the genetic profile or other molecular biomarkers. This demands state-of-the-art diagnostic and prognostic tools, comprehensive molecular characterization of the tumor, as well as detailed electronic patient health records [110].

Computational tools offer the possibility of identifying new entities in signaling cascades as biomarkers and promising targets for anticancer therapy. For example, the Human Protein Atlas provides data on the distribution and the expression of putative gene products in normal and cancer tissues based on immunohistochemical images annotated by pathologists. This database provides cancer protein signatures to be analysed for potential biomarkers [111, 112].

A different approach to the discovery of potential signaling targets is described by metabolomic profiling of biological systems which has been applied to find novel biomarkers for detection and prognosis of the disease [113–115].

Moreover, computational cancer biology and pharmacogenomics have been used for gene targeting by drug repositioning [116, 117]. Computational drug repositioning is another example for *in silico* cancer research, by identifying novel use for FDA-approved drugs, based on available genomic, phenotypic data with the help of bioinformatics and chemoinformatics [118–120]. Computer-aided drug discovery and development have improved the efficiency of pharmaceutical research and link virtual

screening methods, homology and molecular modeling techniques [121, 122]. Pharmacological modeling of drug exposures helps to understand therapeutic exposure-response relationships [123]. Systems pharmacology integrates pharmacokinetic and pharmacodynamic drug relations into the field of systems biology regarding the multiscale physiology [124]. The discipline of pharmacometrics advances to personalized therapy by linking drug response modeling and health records [125]. Polypharmacological effects of multi-drug therapies render exclusive wet lab experimentation unfeasible and require modeling frameworks such as system-level networks [126]. Network pharmacology models involve phenotypic responses and side effects due to a multi-drug treatment, offering information on inhibition, resistance and on-/off-targeting. Moreover, the network approach allows to understand variations within a single cancer disease regarding heterogeneous patient profiles, and in the process, to classify cancer subtypes and to identify novel drug targets [81].

Tumorigenesis is induced by driver mutations and embeds passenger mutations that both can result in upstream or downstream dysregulated signaling pathways [127]. Computational methods have been used to distinguish driver and passenger mutations in cancer pathways by using public genomic databases available through collaborative projects such as the International Cancer Genome Consortium or The Cancer Genome Atlas (TCGA) [62] and others [128], together with functional network analysis using *de novo* pathway learning methods or databases on known pathways such as Gene Ontology [129], Reactome [130] or the Kyoto Encyclopedia of Genes and Genomes (KEGG) [131–134]. These primary pathway databases, based on manually curated physical and functional protein interaction data, are essential for annotation and enrichment analysis. To increase proteome coverage of such analyses, pathways can be integrated with comprehensive protein-protein interaction data and data mining approaches to predict novel, functional protein: pathway associations [135]. Importantly, this *in silico* approach not only expands information on already known parts of the proteome, it also annotates current “pathway orphans” such as proteins that currently do not have any known pathway association.

Comprehensive preclinical models on molecular features of cancer and diverse therapeutic responses have been built as pharmacogenomic resource for precision oncology [136, 137]. Future efforts will need to expand integrative approaches to combine information on multiple levels of molecular aberrations in DNA, RNA, proteins and epigenetic factors [62, 138], as well as cellular aspects of the microenvironment and tumor purity [139], in order to extend treatment efficacy and further refine precision medicine.

Conclusion

Informatics in aid to biomedical research, especially in the field of cancer research, faces the challenge of an overwhelming amount of available data, especially in future regards to personalized medicine [140]. Computational biology provides mathematical models and specialized algorithms to study and predict events in biological systems [141]. Certainly, biomedical researchers from diverse fields will require computational tools in order to better integrate, annotate, analyze, and extract knowledge from large networks of biological systems. This increasing need of understanding complex systems can be supported by “Executable Biology” [142], which embraces representative computational modeling of biological systems.

There is an evolution towards computational cancer research. In particular, *in silico* methods have been suggested for refining experimental programs of clinical and general biomedical studies involving laboratory work [143]. The principles of the 3Rs can be applied to cancer research for the reduction of animal research, saving resources as well as reducing costs spent on clinical and wet lab experiments. Computational modeling and simulations offer new possibilities for research. Cancer and biomedical science in general will benefit from the combination of *in silico* with *in vitro* and *in vivo* methods, resulting in higher specificity and speed, providing more accurate, more detailed and refined models faster. *In silico* cancer models have been proposed as refinement [143]. We further suggest the combination of *in silico* modeling and human computer interaction for knowledge discovery, gaining new insights, supporting prediction and decision making [144].

Here, we provided some thoughts as a motivator for fostering *in silico* modeling towards 3R, in consideration of refinement of testing methods, and gaining a better understanding of tumorigenesis as tumor promotion, progression and dynamics.

Abbreviations

3R: Refinement, reduction, replacement; AOP: Adverse outcome pathway; CAM: Chorioallantoic membrane; ECM: Extracellular matrix; FDA: Food and drug administration; KEGG: Kyoto encyclopedia of genes and genomes; pathDIP: Pathway data integration portal; QSAR: Quantitative structure-activity relationship; REACH: Registration, evaluation, authorisation and restriction of chemicals; SCID: Severe combined immune deficiency; TCGA: The cancer genome atlas; WHO: World health organization

Acknowledgements

This work is based on research studies by students and members of the Holzinger group.

Funding

No specific funding was received for this study.

Availability of data and materials

The data-sets used and/or analysed during the current study are available from the corresponding author on reasonable request and data sources are referenced within figure legend and document text. Images were created by

FJ and CJ under the terms of the Creative Commons license, stated in the Open Access subsection of Declarations. Figure 3 makes use of free to reuse pictures from pixabay and wikimedia licensed under Creative Commons and being free of known restrictions under copyright law, including all related and neighboring rights.

Authors' contributions

CJ initiated and conceived the study, wrote the manuscript and contributed with biochemical expertise. FJ participated in the drafting of the manuscript and contributed with expertise in Informatics related to *in silico* modeling. IJ reviewed and refined the manuscript with expertise in integrative computational biology and cancer informatics. AH supervised the project and contributed with expertise in health informatics. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Holzinger Group, Institute for Medical Informatics, Statistics and Documentation, Medical University Graz, Graz, Austria. ²Institute of Interactive Systems and Data Science, Graz University of Technology, Graz, Austria. ³Krembil Research Institute, University Health Network; Depts. of Medical Bioph. and Comp. Sci., University of Toronto; Institute of Neuroimmunology, Slovak Academy of Sciences, Toronto, Canada.

Received: 29 March 2017 Accepted: 26 March 2018

Published online: 12 April 2018

References

- World Health Organization. Cancer Fact sheet. Updated February 2017. <http://www.who.int/mediacentre/factsheets/fs297/en/>. Accessed Mar 2017.
- National Cancer Institute. Cancer Statistics at a Glance. <https://www.cancer.gov/about-cancer/understanding/statistics>. Last updated Dec 2015. Accessed Mar 2017.
- Wagoner J. Occupational carcinogenesis: the two hundred years since percellvill pott. *Ann NY Acad Sci*. 1976;271(1):1–4.
- Stadler M, Walter S, Walzl A, Kramer N, Unger C, Scherzer M, Unterleuthner D, Hengstschlager M, Krupitza G, Dolznig H. Increased complexity in carcinomas: Analyzing and modeling the interaction of human cancer cells with their microenvironment. *Semin Cancer Biol*. 2015;35:107–24.
- Schwartz AS, Yu J, Gardenour KR, Finley RLJ, Ideker T. Cost-effective strategies for completing the interactome. *Nat Methods*. 2009;6:55–61. Bundesministerium für Wissenschaft, Forschung und Wirtschaft. Tierversuchstatistik 2015. <https://bmbwf.gv.at/das-ministerium/publikationen/forschung/statistiken/tierversuchstatistiken>. Accessed Nov 2017.
- Senatskommission für tierexperimentelle Forschung der Deutschen Forschungsgemeinschaft. *Tierversuche in der Forschung*. Bonn: Deutsche Forschungsgemeinschaft; 2016. http://www.dfg.de/download/pdf/dfg_im_profil/geschaeftsstelle/publikationen/tierversuche_forschung.pdf.
- Canadian Council on Animal Care. CCAC 2016 Animal Data Report. Ottawa: CCAC; 2016. <https://www.ccac.ca/Documents/AUD/2016-Animal-Data-Report.pdf>. Accessed Mar 2018.
- U.K. Government. Annual Statistics of Scientific Procedures on Living Animals Great Britain. London: Williams Lea Group; 2016. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/627284/annual-statistics-scientificprocedures-living-animals-2016.pdf. Accessed Mar 2018.

10. Animal and Plant Health Inspection Service. Annual Report Animal Usage by Fiscal Year: United States Department of Agriculture; 2016. Updated August 2016, Accessed March 2018. https://www.aphis.usda.gov/animal_welfare/downloads/reports/Annual-Report-Animal-Usage-by-FY2016.pdf.
11. Leary SA. The Exclusion of Mice, Rats, and Birds: The American Anti-Vivisection Society; 2017, pp. 12–13. http://aavs.org/assets/uploads/2017/08/2017-1_av-magazine_exclusion-mice-rats-birds.pdf?x82509.
12. MacLaughlin K. China finally setting guidelines for treating lab animals. 2016. <https://doi.org/10.1126/science.aaf9812>. <http://www.sciencemag.org/news/2016/03/china-finally-setting-guidelines-treating-lab-animals>. Accessed Feb 2017.
13. Humane Research Australia. Australian Statistics of Animal Use in Research and Teaching. 2015. http://www.humanereseach.org.au/statistics/statistics_2015. Accessed Mar 2018.
14. Bundesministerium für Ernährung und Landwirtschaft. Verwendung von Versuchstieren im Jahr 2016. 2016. https://www.bmel.de/DE/Tier/Tierschutz/_texte/TierschutzTierforschung.html?docId=10323474. Accessed Mar 2018.
15. Russell W, Burch R. The Principles of Humane Experimental Technique. Reprinted 1992 ed. UK: Wheathampstead: Universities Federation for Animal Welfare; 1959.
16. Latham SR. U.S. law and animal experimentation: A critical primer. *Hast Cent Rep*. 2012;42(6):35–9.
17. Tannenbaum J, Rowan AN. Rethinking the morality of animal research. *Hast Cent Rep*. 1985;15:32–43.
18. Sneddon LU, Halsey LG, Bury NR. Considering aspects of the 3rs principles within experimental animal biology. *J Exp Biol*. 2017;220(17):3007–16. <https://doi.org/10.1242/jeb.147058>.
19. Morrissey B, Blyth K, Carter P, Chelala C, Jones L, Holen I, Speirs V. The sharing experimental animal resources, coordinating holdings (search) framework: Encouraging reduction, replacement, and refinement in animal research. *PLoS Biol*. 2017;15(1):2000719. <https://doi.org/10.1371/journal.pbio.2000719>.
20. Benfenati E, Benign R, DeMarini DM, Helma C, Kirkland D, Martin TM, Mazzatorta P, Ouédraogo-Arras G, Richard AM, Schilter B, Schoonen WGEJ, Snyder RD, Yang C. Predictive models for carcinogenicity and mutagenicity: Frameworks, state-of-the-art, and perspectives. *J Environ Sci Health C*. 2009;27:57–90.
21. Kleinstreuer NC, Yang J, Berg EL, Knudsen T, Richard AM, Martin M, Reif DM, Judson R, Polokoff M, Dix DJ, et al. Phenotypic screening of the toxcast chemical library to classify toxic and therapeutic mechanisms. *Nat Biotechnol*. 2014;32(6):583–91.
22. Huang R, Xia M, Sakamuru S, Zhao J, Shahane SA, Attene-Ramos M, Zhao T, Austin CP, Simeonov A. Modelling the tox21 10 k chemical profiles for in vivo toxicity prediction and mechanism characterization. *Nat Commun*. 2016;7:10425.
23. Tritthart HA. In vitro test systems in cancer research. *ALTEX*. 1996;13(3):118–24.
24. Teicher BA. In vivo/ex vivo and in situ assays used in cancer research: a brief review. *Toxicol Pathol*. 2009;37(1):114–22.
25. Esch EW, Bahinski A, Huh D. Organs-on-chips at the frontiers of drug discovery. *Nat Rev Drug Discov*. 2015;14:248–60.
26. Viceconti M HP. The virtual physiological human: Ten years after. *Annu Rev Biomed Eng*. 2016;18:103–23.
27. Zurlo J, Rudacille D, Goldberg AM. The three R's: The way forward. *Environ Health Perspect*. 1996;104(8):878.
28. Raunio H. In silico toxicology - non-testing methods. *Front Pharmacol*. 2011;2(33):33.
29. European Chemicals Agency. Guidance on Information Requirements and Chemical Safety Assessment. 2008. <http://echa.europa.eu/>.
30. European Chemicals Agency. Guidance on Information Requirements and Chemical Safety Assessment. 2011. <http://echa.europa.eu/>.
31. Raies AB, Bajic VB. In silico toxicology: Computational methods for the prediction of chemical toxicity. *Wiley Interdiscip Rev Comput Mol Sci*. 2016;6(2):147–72.
32. Workman P, Aboagye E, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double JA, Everitt J, Farningham DAH, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA, ad hoc committee of the National Cancer Research Institute. Guidelines for the welfare and use of animals in cancer research. *Br J Cancer*. 2010;102:1555–77.
33. Klein S, Maggioni S, Bucher J, Mueller D, Niklas J, Shevchenko V, Mauch K, Heinzele E, Noor F. In silico modeling for the prediction of dose and pathway-related adverse effects in humans from in vitro repeated-dose studies. *Toxicol Sci*. 2015;149:55–66.
34. Yee NS, Ignatenko N, Finnberg N, Lee N, Stairs D. Animal models of cancer biology. *Cancer Growth Metastasis*. 2015;8(Suppl 1):115–8.
35. House CD, Hernandez L, Annunziata CM. Recent technological advances in using mouse models to study ovarian cancer. *Front Oncol*. 2014;4(26):26.
36. Kersten K, de Visser KE, van Miltenburg MH, Jonkers J. Genetically engineered mouse models in oncology research and cancer medicine. *EMBO Mol Med*. 2017;9(2):137–53.
37. Gengenbacher N, Singhal M, Augustin HG. Preclinical mouse solid tumour models: status quo, challenges and perspectives. *Nat Rev Cancer*. 2017;17:751–65.
38. Vitale G, Gaudenzi G, Circelli L, Manzoni M, Bassi A, Fioritti N, Faggiano A, Colao A, NIKE group. Animal models of medullary thyroid cancer: state of the art and view to the future. *Endocr Relat Cancer*. 2017;24(1):1–12.
39. Rubio-Viqueira B, Hidalgo M. Direct in vivo xenograft tumor model for predicting chemotherapeutic drug response in cancer patients. *Clin Pharmacol Ther*. 2009;85:217–21.
40. Kariyil BJ. In vivo models in cancer research. *Int J Curr Res*. 2015;7:24399–404.
41. Yadav AK, Srikrishna S, Gupta SC. Cancer drug development using drosophila as an in vivo tool: From bedside to bench and back. *Trends Pharmacol Sci*. 2016;37:789–806.
42. Astone M, Dankert EN, Alam SKK, Hoepfner LH. Fishing for cures: The allure of using zebrafish to develop precision oncology therapies. *npj Precis Oncol*. 2017;1(39):39.
43. Deryugina EIA, Quigley JP. Chick embryo chorioallantoic membrane model systems to study and visualize human tumor cell metastasis. *Histochem Cell Biol*. 2008;130(6):1119–30.
44. Kain KH, Miller JW, Jones-Paris CR, Thomason RT, Lewis JD, Bader DM, Barnett JV, Zijlstra A. The chick embryo as an expanding experimental model for cancer and cardiovascular research. *Dev Dyn*. 2014;243(2):216–28.
45. Gould SE, Junttila MR, de Sauvage FJ. Translational value of mouse models in oncology drug development. *Nat Med*. 2015;21:431–9.
46. Katt ME, Placone AL, Wong AD, Xu ZS, Searson PC. In vitro tumor models: Advantages, disadvantages, variables, and selecting the right platform. *Front Bioeng Biotechnol*. 2016;4:12.
47. Cavanaugh H, Haier J. *Basic Tissue and Cell Culture in Cancer Research*. NY: Wiley; 2005. Chap. Preclinical Methods for Human Cancer.
48. Justus CR, Leffler N, Ruiz-Echevarria M, Yang LV. In vitro cell migration and invasion assays. *Journal of visualized experiments. JoVE*. 2014;88:e51046.
49. Raghavan S, Mehta P, Horst EN, Ward MR, Rowley KR, Mehta G. Comparative analysis of tumor spheroid generation techniques for differential in vitro drug toxicity. *Oncotarget*. 2016;7(13):16948–61.
50. Lee H, Park W, Ryu H, Jeon NL. A microfluidic platform for quantitative analysis of cancer angiogenesis and intravasation. *Biomicrofluidics*. 2014;8(5):054102.
51. Wong AD, Searson PC. Mitosis-mediated intravasation in a tissue-engineered tumor-microvessel platform. *Cancer Res*. 2017;77(22):6453–61.
52. Jijakli K, Khraiwesh B, Fu W, Luo L, Alzhami A, Koussa J, Chaiboonchoe A, Kirmizialtin S, Yen L, Salehi-Ashtiani K. The in vitro selection world. *Methods*. 2016;106:3–13.
53. Pouliot N, Pearson HB, Burrows A. Investigating metastasis using in vitro platforms. *Landes Biosci*. 2013. <http://www.ncbi.nlm.nih.gov/books/NBK100379/>.
54. European Food Safety Authority. Modern methodologies and tools for human hazard assessment of chemicals. *EFSA J*. 2014;12(3638):87.
55. Lovitt CJ, Shelper TB, Avery VM. Advanced cell culture techniques for cancer drug discovery. *Biology*. 2014;3:345–67.
56. Ahamer H, Devaney TJJ, Tritthart HA. Fractal dimension for a cancer invasion model. *Fractals Compl Geom Patt Scaling Nat Soc*. 2001;9(01):61–76.
57. Turin I, Schiavo R, Maestri M, Luinetti O, Bello BD, Paulli M, Dionigi P, Roccio M, Spinillo A, Ferulli F, Tanzi M, Maccario R, Montagna D, Pedrazzoli P. In vitro efficient expansion of tumor cells deriving from different types of human tumor samples. *Med Sci*. 2014;2(2):70–81.

58. Mackenzie I. New in vitro assays for studying the biology of cancer stem cells. In: NC3Rs research portfolio. London: National Centre for the Replacement Refinement & Reduction of Animals in Research; 2011. p. 37–40. https://www.nc3rs.org.uk/sites/default/files/documents/Corporate_publications/Research_Reviews/Research%20Review%20Review%20Review.pdf.
59. Vaira V, Fedele G, Pyne S, Fasoli E, Zadra G, Bailey D, Snyder E, Favarsani A, Coggi G, Flavin R, Bosari S, Loda M. Preclinical model of organotypic culture for pharmacodynamic profiling of human tumors. *PNAS*. 2010;107(18):8352–8356.
60. Aref A, Barbie D. *Ex Vivo Engineering of the Tumor Microenvironment*. Berlin: Springer; 2017. p. 135.
61. Jeanquartier F, Jean-Quartier C, Schreck T, Cemernek D, Holzinger A. Integrating open data on cancer in support to tumor growth analysis. *Inf Tech Bio Med Inf Lect Notes Comput Sci Lect Notes Comput Sci LNCS*. 2016;9832(Information Technology in Bio- and Medic):49–66.
62. Weinstein JN, Collisson EA, Mills GB, Shaw KM, Ozenberger BA, Ellrott K, Cancer Genome Atlas Research Network. The cancer genome atlas pan-cancer analysis project. *Nat Genet*. 2013;45(10):1113–20.
63. Riggins G J SRL. Genome and genetic resources for the cancer genome anatomy project. *Hum Mol Genet*. 2001;7:663–7.
64. Subramanian A, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, Gould J, Davis JF, Tubelli AA, Asiedu JK, Lahr DL, Hirschman JE, Liu X, Donahue M, Julian B, Khan M, Wadden D, Smith IC, Lam D, Liberzon A, Toder C, Bagul M, Orzechowski M, Enache OM, Piccioni F, Johnson SA, Lyons NJ, Berger AH, Shamji AF, Brooks AN, Vrcic A, Flynn C, Rosains J, Takeda DY, Hu R, Davison D, Lamb J, Ardlie K, Hogstrom L, Greenside P, Gray NS, Clemons PA, Silver S, Wu X, Zhao W-N, Read-Button W, Wu X, Haggarty SJ, Ronco LV, Boehm JS, Schreiber SL, Doench JG, Bittker JA, Root DE, Wong B, Golub TR. A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. *Cell*. 2017;171(6):1437–1452.e17. <https://doi.org/10.1016/j.cell.2017.10.049>.
65. The Humane Society of the United States and Procter & Gamble. AltTox: Toxicity testing overview. Last updated: February 19, 2016. <http://alttox.org/mapp/toxicity-testing-overview/>. Accessed Mar 2017.
66. Judson R, Houck K, Martin M, Knudsen T, Thomas RS, Sipes N, Shah I, Wambaugh J, Crofton K. In vitro and modelling approaches to risk assessment from the us environmental protection agency toxcast programme. *Basic Clin Pharmacol Toxicol*. 2014;115(1):69–76.
67. Igarashi Y, Nakatsu N, Yamashita T, Ono A, Ohno Y, Urushidani T, Yamada H. Open tg-gates: a large-scale toxicogenomics database. *Nucleic Acids Res*. 2015;43(Database issue):921–7.
68. Mulas F, Li A, Sherr DH, Monti S. Network-based analysis of transcriptional profiles from chemical perturbations experiments. *BMC Bioinformatics*. 2017;18(Suppl 5):130.
69. Hofree M, Carter H, Kreisberg JF, Bandyopadhyay S, Mischel PS, Friend S, Ideker T. Challenges in identifying cancer genes by analysis of exome sequencing data. *Nat Commun*. 2016;7:12096.
70. Handl J, Knowles J, Kell DB. Computational cluster validation in post-genomic data analysis. *Bioinformatics*. 2005;21(15):3201–12.
71. Kaufman L, Rousseeuw PJ. *Finding Groups in Data: an Introduction to Cluster Analysis*, vol 344. NY: Wiley; 2009.
72. Geman D, Ochs M, Price ND, Tomasetti C, Younes L. An argument for mechanism-based statistical inference in cancer. *Hum Genet*. 2015;134(5):479–95.
73. Sur I, Taipale J. The role of enhancers in cancer. *Nat Rev Cancer*. 2016;16(8):483–93.
74. Piraino SW, Furney SJ. Beyond the exome: the role of non-coding somatic mutations in cancer. *Ann Oncol*. 2016;27(2):240–8.
75. Cuykendall TN, Rubin MA, Khurana E. Non-coding genetic variation in cancer. *Curr Opin Syst Biol*. 2017;1(Supplement C):9–15. *Future of Systems Biology, Genomics and epigenomics*.
76. Morrow JJ, Scacheri PC. Epigenetics, enhancers, and cancer. In: Berger NA, editor. *Energy Balance and Cancer*. Switzerland: Springer; 2016. p. 29–53.
77. Medvedeva YA, Lennartsson A, Ehsani R, Kulakovskiy IV, Vorontsov IE, Panahandeh P, Khimulya G, Kasukawa T, FANTOM Consortium, Drabli A S. F. Epifactors: a comprehensive database of human epigenetic factors and complexes. *Database*. 2015;2015:bav067.
78. Johnson C, Warmoes MO, Shen X, Locasale JW. Epigenetics and cancer metabolism. *Cancer Lett*. 2015;356(2):209–314.
79. Heyn H, Vidal E, Ferreira HJ, Vizoso M, Sayols S, Gomez A, Moran S, Boque-Sastre R, Guil S, Martinez-Cardus A, Lin CY, Royo R, Sanchez-Mut JV, Martinez R, Gut M, Torrents D, Orozco M, Gut I, Young RA, Esteller M. Epigenomic analysis detects aberrant super-enhancer DNA methylation in human cancer. *Genome Biol*. 2016;17:11.
80. Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. *Nat Rev Cancer*. 2010;10:389–402.
81. Kim Y-A, Cho D-Y, Przytycka TM. Understanding genotype-phenotype effects in cancer via network approaches. *PLoS Comput Biol*. 2016;12(3):e1004747.
82. Porta-Pardo E, Garcia-Alonso L, Hrabe T, Dopazo J, Godzik A. A pan-cancer catalogue of cancer driver protein interaction interfaces. *PLOS*. 2015;11:e1004518.
83. Maximova T, Mofatt R, Ma B, Nussinov R, Shehu A. Principles and overview of sampling methods for modeling macromolecular structure and dynamics. *PLOS Comput Biol*. 2016;12:e1004619.
84. Smolle J, Hofmann-Wellenhof R, Kofler R, Cerroni L, Haas J, Kerl H. Computer simulations of histologic patterns in melanoma using a cellular automaton provide correlations with prognosis. *J Investig Dermatol*. 1995;105:797–801.
85. Patil SA, Kuchanur MB. Lung cancer classification using image processing. *Int J Eng Innov Technol*. 2012;2(30):37–42.
86. Vargas A, Angeli M, Pastrello C, McQuaid R, Li H, Jurisica I. Robust quantitative scratch assay. *Bioinformatics*. 2016;2(9):1439–40.
87. Lu W, Wang J, Zhang HH. Computerized PET/CT image analysis in the evaluation of tumour response to therapy. *Br J Radiol*. 2015;88(1048):20140625–20140625.
88. Tiwari S, Bhargava R. Extracting knowledge from chemical imaging data using computational algorithms for digital cancer diagnosis. *Yale J Biol Med*. 2015;88:131–43.
89. Christopher R, Dhiman A, Fox J, Gendelman R, Haberitcher T, Kagle D, Spizz G, Khalil I, Hill C. Data-driven computer simulation of human cancer cell. *Ann N Y Acad Sci*. 2004;1020:132–53.
90. Nilsson A, Nielsen J. Genome scale metabolic modeling of cancer. *Metab Eng*. 2016. <https://doi.org/10.1016/j.ymben.2016.10.022>.
91. Beerenwinkel N, Greenman CD, Lagergren J. Computational cancer biology: An evolutionary perspective. *PLoS Comput Biol*. 2016;12(2):1004717.
92. Benzekry S, Lamont C, Beheshti A, Tracz A, Ebos JM, Hlatky L, Hahnfeldt P. Classical mathematical models for description and prediction of experimental tumor growth. *PLoS Comput Biol*. 2014;10(8):1003800.
93. Rejniak KA, Anderson AR. Hybrid models of tumor growth. *Wiley Interdiscip Rev Syst Biol Med*. 2011;3(1):115–25.
94. Kohonen P, Ceder R, Smit I, Hongisto V, Myatt G, Hardy B, Spjuth O, Grafström R. Cancer biology, toxicology and alternative methods development go hand-in-hand. *Basic Clin Pharmacol Toxicol*. 2014;115:50–8.
95. Langley GR, Adcock IM, Busquet F, Crofton KM, Csernok E, Giese C, Heinonen T, Herrmann K, Hofmann-Apitius M, Landesmann B, et al. Towards a 21st-century roadmap for biomedical research and drug discovery: consensus report and recommendations. *Drug Discov Today*. 2017;22(2):327–39.
96. Organisation de Coopération et de Développement Économiques. Series on testing and assessment: Guidance document on developing and assessing adverse outcome pathways. Technical Report 184, Environment, OECD, Health and Safety Publications. 2017.
97. Perkins EJ, Antczak P, Burgoon L, Falciani F, Garcia-Reyer N, Gutsell S, Hodges G, Kienzler A, Knapen D, McBride M, Willett C. Adverse outcome pathways for regulatory applications: Examination of four case studies with different degrees of completeness and scientific confidence. *Toxicol Sci*. 2015;148:14–25.
98. Boobis AR, Cohen SM, Dellarco V, McGregor D, Vickers MEBMC, Willcocks D, Farland W. Ipc framework for analyzing the relevance of a cancer mode of action for humans. *Crit Rev Toxicol*. 2006;36:781–92.
99. Kotlyar M, Fortney K, Jurisica I. Network-based characterization of drug-regulated genes, drug targets, and toxicity. *Methods*. 2012;57(4):499–507.
100. Fjodorova N, Vračko M, Novič M, Roncaglioni A, Benfenati E. New public QSAR model for carcinogenicity. *Chem Cent J*. 2010;4(supplement 1):S3.
101. Malik A, Singh H, Andrabi M, Husain SA, Ahmad S. Databases and qsar for cancer research. *Cancer Informat*. 2006;2:99–111.
102. Menden M, Iorio F, Garnett M, McDermott U, Benes C, Ballester PJ, Saez-Rodriguez J. Machine learning prediction of cancer cell sensitivity

- to drugs based on genomic and chemical properties. *PLoS ONE*. 2013;8(4):61318.
103. Ghersi D, Singh M. Interaction-based discovery of functionally important genes in cancers. *Nucl Acids Res*. 2014;42(3):e18.
 104. Yang W, Soares J, Greninger P, Edelman EJ, Lightfoot H, Forbes S, Bindal N, Beare D, Smith JA, Thompson IR, Ramaswamy S, Futreal PA, Haber D, Stratton M, Benes C, McDermott U, Garnett M. Genomics of drug sensitivity in cancer (gdscc): a resource for therapeutic biomarker discovery in cancer cells. *Nucleic Acids Res*. 2013;41(Database issue):955–61.
 105. Villaverde AF, Henriques D, Smallbone K, Bongard S, Schmid J, Cicin-Sain D, Crombach A, Saez-Rodriguez J, Mauch K, Balsa-Canto E, Mendes P, Jaeger J, Banga JR. Biopredyn-bench: a suite of benchmark problems for dynamic modelling in systems biology. *BMC Syst Biol*. 2015;9:8.
 106. Hochheiser H, Castine M, Harris D, Savova G, Jacobson RS. An information model for computable cancer phenotypes. *BMC Med Inform Decis Mak*. 2016;16(1):121.
 107. Jeanquartier F, Jean-Quartier C, Kotlyar M, Tokar T, Hauschild A-C, Jurisica I, Holzinger A. Machine learning for in silico modeling of tumor growth. In: *Machine Learning for Health Informatics*. Cham: Springer; 2016. p. 415–34.
 108. Gonçalves E, Bucher J, Ryll A, Niklas J, Mauch K, Klamt S, Rocha M, Saez-Rodriguez J. Bridging the layers: Towards integration of signal transduction, regulation and metabolism into mathematical models. *Mol BioSyst*. 2013;9:1576–83.
 109. Carels N, Spinassé LB, Tilli TM, Tuszyński JA. Toward precision medicine of breast cancer. *Theor Biol Med Model*. 2016;13:7.
 110. Mirnezami R, Nicholson J, Darzi A. Preparing for precision medicine. *N Engl J Med*. 2012;366:489–91.
 111. Uhlén M, Björling E, Agaton C, Szgyarto C, Amini B, Andersen E, Andersson A, Angelidou P, Asplund A, Asplund C, Berglund L, Bergström K, Brumer H, Cerjan D, Ekström M, Eloheid A, Eriksson C, Fagerberg L, Falk R, Fall J, Forsberg M, Björklund M, Gumbel K, Halimi A, Hallin I, Hamsten C, Hansson M, Hedhammar M, Hercules G, Kampf C, Larsson K, Lindskog M, Lodewyckx W, Lund J, Lundeberg J, Magnusson K, Malm E, Nilsson P, Odling J, Oksvold P, IO, E O, J O, L P, A P, Rimini R, Rockberg J, Runeson M, Sivertsson A, Sköllerö A, J S, M S, Sterky F, Strömberg S, Sundberg M, Tegel H, Tourle S, Wahlund E, Waldén A, Wan J, Wernérus H, Westberg J, Wester K, U W, Xu L, Hober S, Pontén F. A human protein atlas for normal and cancer tissues based on antibody proteomics. *Mol Cell Proteomics*. 2005;4(12):1920–32.
 112. Uhlen M, Zhang C, Lee S, Sjöstedt E, Fagerberg L, Bidkhorji G, Benfaisat R, Arif M, Liu Z, Edfors F, Sanli K, von Feilitzen K, Oksvold P, Lundberg E, Hober S, Nilsson P, Mattsson J, Schwenk JM, Brunström H, Glimelius B, Sjöblom T, Edqvist P-H, Djureinovic D, Micke P, Lindskog C, Mardinoglu A, Ponten F. A pathology atlas of the human cancer transcriptome. *Sci (New York, NY)*. 017;357(6352). <https://doi.org/10.1126/science.aan2507>.
 113. Locasale JW, Melman T, Song S, Yang X, Swanson KD, Cantley LC, Wong ET, Asara JM. Metabolomics of human cerebrospinal fluid identifies signatures of malignant glioma. *Mol Cell Proteomics*. 2012;11(6):M111.014688.
 114. McDunn JE, Li Z, Adam K-P, Neri BP, Wolfert RL, Milburn MV, Lotan Y, Wheeler TM. Metabolomic signatures of aggressive prostate cancer. *Prostate*. 2013;73(73):1547–60.
 115. Farshidfar F, Weljie AM, Kopciuk KA, Hilsden R, McGregor SE, Buie WD, MacLean A, Vogel HJ, Bathe OF. A validated metabolomic signature for colorectal cancer: exploration of the clinical value of metabolomics. *Br J Cancer*. 2016;115:848–57.
 116. Belizário JE, Sanguiliano BA, Perez-Sosa M, Neyra JM, Moreira DF. Using pharmacogenomic databases for discovering patient-target genes and small molecule candidates to cancer therapy. *Front Pharmacol*. 2016;7:312.
 117. Fortney K, Griesman J, Kotlyar M, Pastrello C, Angeli M, Sound-Tsao M, Jurisica I. Prioritizing therapeutics for lung cancer: An integrative meta-analysis of cancer gene signatures and chemogenomic data. *PLoS Comput Biol*. 2015;11(3):e1004068.
 118. Shim JS LJ. Recent advances in drug repositioning for the discovery of new anticancer drugs. *Int J Biol Sci*. 2014;10(7):654–63.
 119. Jiao M, Liu G, Xue Y, Ding C. Computational drug repositioning for cancer therapeutics. *Curr Top Med Chem*. 2015;15(8):767–75.
 120. Li J, Zheng S, Chen B, Butte A, Swamidass S, Lu Z. A survey of current trends in computational drug repositioning. *Brief Bioinform*. 2016;17(1):2–12.
 121. Zhang S. Computer-aided drug discovery and development. *Methods Mol Biol*. 2011;716:23–38.
 122. Guedes RA, Serra P, Salvador JA, Guedes RC. Computational approaches for the discovery of human proteasome inhibitors: An overview. *Molecules*. 2016;21(7):927.
 123. Yamazaki S, Spilker M, Vicini P. Translational modeling and simulation approaches for molecularly targeted small molecule anticancer agents from bench to bedside. *Expert Opin Drug Metab Toxicol*. 2016;12(3):253–6.
 124. Li X, Oduola W, Qian L, Dougherty E. Integrating multiscale modeling with drug effects for cancer treatment. *Cancer Informat*. 2016;14(Suppl 5):21–31.
 125. Buil-Bruna N, López-Picazo J, Martín-Algarra S, Trocóniz I. Bringing model-based prediction to oncology clinical practice: A review of pharmacometrics principles and applications. *Oncologist*. 2016;21(2):220–32.
 126. Tang J, Aittokallio T. Network pharmacology strategies toward multi-target anticancer therapies: from computational models to experimental design principles. *Curr Pharm Des*. 2014;20(1):23–36.
 127. Nussinov R, Jang H, Tsai C-J. The structural basis for cancer treatment decisions. *Oncotarget*. 2014;5(17):7285–302.
 128. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C, Schultz N. The cbio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Disc*. 2012;2(5):401–4. <https://doi.org/10.1158/2159-8290.cd-12-0095>.
 129. The Gene Ontology Consortium. Expansion of the gene ontology knowledgebase and resources. *Nucleic Acids Res*. 2017;45(D1):331–8. <https://doi.org/10.1093/nar/gkw1108>.
 130. Fabregat A, Jupe S, Matthews L, Sidiropoulos K, Gillespie M, Garapati P, Haw R, Jassal B, Korninger F, May B, Milacic M, Roca CD, Rothfels K, Sevilla C, Shamovsky V, Shorsler S, Varusai T, Viteri G, Weiser J, Wu G, Stein L, Hermjakob H, D'Eustachio P. The reactome pathway knowledgebase. *Nucleic Acids Res*. 2017. <https://doi.org/10.1093/nar/gkx1132>.
 131. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. Kegg: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res*. 2017;45(D1):353–61. <https://doi.org/10.1093/nar/gkw1092>.
 132. Merid SK, Goranskaya D, Alexeyenko A. Distinguishing between driver and passenger mutations in individual cancer genomes by network enrichment analysis. *BMC Bioinformatics*. 2014;15:308.
 133. Dimitrakopoulos CM, Beerenwinkel N. Computational approaches for the identification of cancer genes and pathways. *Wiley Interdiscip Rev Syst Biol Med*. 2016;9(1):e1364.
 134. Leiserson MDM, Vandin F, Wu H-T, Dobson JR, Eldridge JV, Thomas JL, Papoutsaki A, Kim Y, Niu B, McLellan M, Lawrence MS, Gonzalez-Perez A, Tamborero D, Cheng Y, Ryslik GA, Lopez-Bigas N, Getz G, Ding L, Raphael BJ. Pan-cancer network analysis identifies combinations of rare somatic mutations across pathways and protein complexes. *Nat Genet*. 2015;47(2):106–14.
 135. Rahmati S, Abovsky M, Pastrello C, Jurisica I. pathDIP: an annotated resource for known and predicted human gene-pathway associations and pathway enrichment analysis. *Nucleic Acids Res*. 2017;45(D1):D419–D426.
 136. Iorio F, Knijnenburg T, Vis D, Bignell G, Menden M, Schubert M, Aben N, Gonçalves E, Barthorpe S, Lightfoot H, Cokelaer T, Greninger P, van Dyk E, Chang H, de Silva H, Heyn H, Deng X, Egan R, Liu Q, Mironenko T, Mitropoulos X, Richardson L, Wang J, Zhang T, Moran S, Sayols S, Soleimani M, Tamborero D, Lopez-Bigas N, Ross-Macdonald P, Esteller M, Gray N, Haber D, Stratton M, Benes C, Wessels L, Saez-Rodriguez J, McDermott U, MJ G. A landscape of pharmacogenomic interactions in cancer. *Cell*. 2016;166:740–54.
 137. Schubert M, Klinger B, Klünemann M, Garnett M, Blüthgen N, Saez-Rodriguez J. Perturbation-response genes reveal signaling footprints in cancer gene expression. *bioRxiv*. 2016;065672. <http://biorxiv.org/content/early/2016/08/28/065672.full.pdf>.
 138. Holzinger I, Jurisica A. Knowledge Discovery and Data Mining in Biomedical Informatics: The Future Is in Integrative, Interactive Machine Learning Solutions Vol. 8401. Heidelberg: Springer; 2014, pp. 1–18.
 139. Aran D, Sirota M, Butte A. Systematic pan-cancer analysis of tumour purity. *Nat Commun*. 2015;4(6):8971.

140. Nass SJ, Wizemann T, et al. Informatics Needs and Challenges in Cancer Research: Workshop Summary. Washington (DC): National Academies Press (US); 2012.
141. Kitano H. Computational systems biology. *Nature*. 2002;420(6912):206–10.
142. Fisher J, Henzinger TA. Executable cell biology. *Nat Biotechnol*. 2007;25(11):1239–49.
143. Trisilowati & Mallet DG. In silico experimental modeling of cancer treatment. *ISRN Oncol*. 2012;2012:828701.
144. Jeanquartier F, Jean-Quartier C, Cemernek D, Holzinger A. In silico modeling for tumor growth visualization. *BMC Syst Biol*. 2016;10:59.
145. Reinhold WC, Sunshine M, Liu H, Varma S, Kohn KW, Morris J, Doroshov J, Pommier Y. Cellminer: a web-based suite of genomic and pharmacologic tools to explore transcript and drug patterns in the nci-60 cell line set. *Cancer Res*. 2012;72(14):3499–511. <https://doi.org/10.1158/0008-5472.can-12-1370>.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

