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Lessons Learned from Two Decades of Anticancer Drugs

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20 Tremendous efforts have been made to elucidate the basis of cancer biology with the aim of
21 promoting anticancer drug development. Especially in the past twenty years, anticancer drug
22 development has developed from conventional cytotoxic agents to target-based and immune-
23 related therapies. Consequently, more than 200 anticancer drugs are available on the market.
24 However, anticancer drug development still suffers high attrition in the later phases of clinical
25 development and is considered to be a difficult and risky therapeutic category within the drug
26 development arena. The disappointing performance of investigational anticancer candidates
27 implies that there are some shortcomings in the translation of preclinical in vitro and in vivo
28 models to humans, and that heterogeneity in the patient population presents a significant
29 challenge. Here, we summarize both successful and failed experiences in anticancer
30 development during the past 20 years and help identify why the paradigm may be suboptimal.
31 We also offer potential strategies for improvement.

32

33 Current progress of anticancer drug development

34 Cancer, which is characterized by uncontrolled growth of cells in the body, is one of the most
35 difficult and complex diseases to treat [1-3]. Cancer patients suffer high mortality rates, which
36 range from 1.1% for prostate cancer to 92.3% for pancreatic cancer within five years after
37 cancer diagnosis. Therefore, anticancer drug research and development (R&D) is a challenging
38 and daunting activity, and the likelihood of failure is high [4]. Fewer than 5% of developed
39 anticancer compounds reach the market [5]. Furthermore, compared to other therapeutic
40 categories such as cardiovascular disease and arthritis, an anticancer drug has approximately
41 one-third to one-half greater failure rate per attempt [5, 6]. Although there are a lot of
42 difficulties and barriers in anticancer drug development, drug makers are still pursuing
43 opportunities for anticancer drug candidates due to their high cost-benefit rate [7, 8]. For
44 example, oncology is ranked in the top therapeutic class by global sales which amounted to
45 78.94 billion US dollars in 2015.

Approved anticancer drugs

The ultimate task for an anticancer drug is to kill the tumor cells and/or control proliferation of tumor cells to prolong patient survival and improve their quality of life. However, there are many different mechanisms by which this can be achieved. Based on the key biochemical mechanism of anticancer action, anticancer drugs can be are categorized as: (*i) nucleic acid biosynthesis blocker;* (ii) *the structure and function of DNA interferer;* (iii) *transcription interferer and RNA synthesis blocker;*(iv) *protein synthesis and function interferer;* (v) *hormone homeostasis influencer*; (vi) *immune system modulators*. Consequently, drug makers have produced four major groups of anticancer drugs including cytotoxic drugs (alkylating agents, antimetabolites, antibiotics, plant extracts, and miscellaneous cytotoxic drugs), targeted-based agents (e.g. bevacizumab), hormones and hormones antagonists (e.g. tamoxifen), and immunomodulators (e.g. nivolumab). From the classic "*Seed and soil hypothesis*" that first described metastasis [9] to the first description of "*immune-based cancer therapy*" [10], every milestone made in the cancer field has driven a wave of anticancer drug development [11] (**Figure 1**). In the past two decades, anticancer drug development has moved on from conventional non-specific cytotoxic agents which often kill proliferating normal cells as well as tumor cells [12, 13]. In the place of cytotoxic agents, there is a focus on specific target-based cancer therapy [14] designed to hit tumor cells only, and on immune-related modulators that help the patient's immune system to defeat tumor cells [10, 15, 16]. Furthermore, a series of regulations, initiatives, and guidance have been developed to facilitate anticancer drug development [17, 18].

According to the USA National Cancer Institute drug repository, there are a total of 227 approved anticancer drugs (Supplementary **Table S1**) to treat about 40 different types of cancers. There are multiple drug options developed for leukemia, non-Hodgkin lymphoma and breast cancers. In contrast, for some cancer types such as penile or liver cancer, there is only one drug treatment available. On average, available anticancer drugs are used to treat 3.44 cancer types. For example, nivolumab (Opdivo®) is a monoclonal antibody that works as a checkpoint inhibitor by inhibiting the programmed cell death receptor 1 (PD-1), which is

74 overexpressed on diverse of tumor cells and is in charge of down-regulating the immune
75 system and suppressing T cell inflammatory activity. Activated PD-1 blocks T-cell activation and
76 aids the tumor in escaping immune detection. By blocking this PD-1 activation, nivolumab aids
77 the immune system in attacking the tumor cells [19, 20]. Nivolumab was initially approved as a
78 first-line anticancer drug to treat advanced melanoma in 2014. In 2015, the indication of
79 nivolumab was expanded to squamous cell lung cancer and as a second line anticancer drug to
80 treat renal cell carcinoma . In addition, nivolumab was also approved in 2016 to treat classical
81 Hodgkin lymphoma (cHL) in patients who have relapsed or progressed after post-
82 transplantation brentuximab vedotin and autologous hematopoietic stem cell transplantation
83 (auto-HSCT) . In contrast, approximately 45% of rare diseases are rare oncological diseases [21],
84 some of which have no treatment options available on the market [22]. There is no obvious
85 correlation between the number of drugs to treat a particular cancer and the five-year survival
86 rate/estimated new cases for different cancer types, which may imply that developments in
87 treatment is mainly based on the understanding of cancer nature history and on our
88 accumulated knowledge on pathogenesis and etiology of cancer (**Figure 2**).

89 **Clinical trials related cancers and other neoplasms**

90 Anticancer drug development remains a major focus of clinical trials and approximately 40% of
91 studies in clinicaltrial.gov are relevant to the condition "*Cancers and Other Neoplasms*" [23].
92 These clinical studies are widely sponsored by drug makers, academic researchers, and federal
93 governments. For example, the NCI has supported or sponsored a total of more than 5000
94 cancer-related clinical trials. Among 5154 cancer-related clinical trials, about 73% (3735/5114)
95 of clinical studies are aimed at developing treatment options for cancers. These cancer
96 treatment-related studies are in different clinical phases with 45% in phase I, 53% in Phase II,
97 8.6% in Phase III and only 0.6% in Phase IV. This shows that although many compounds enter
98 the early phases (I & II), relatively few make it to Phase III or beyond.

99 High failure rate of anticancer drugs

100 Ongoing efforts have uncovered cancer genetics, novel therapeutic targets, and clinical
101 biomarkers related to survival rate, which have led to better understanding of the molecular
102 basis of cancer. However, it seems that our ability to translate these research findings into
103 more effective clinical cancer treatments is still remarkably limited [24, 25]. Many factors are
104 responsible for a high attrition rate of anticancer drug development at each phase from
105 preclinical to post-marketing of drug development.

106 ***In vitro* assay approaches**

107 The key challenge for preclinical in vitro and in vivo tools such as cancer-cell-lines and animal
108 models is whether they can be used to make reliable "go/no go" decisions on which candidates
109 to progress into the clinical phases. Concerns have been raised as to whether cancer-cell-line
110 based assay systems can meaningfully reproduce the tumor cell behaviors in cancer patients.
111 High-throughput screening (HTS) based *In vitro* assays have a lot of advantages since they can
112 be used to conduct a rapid screen of anticancer drug candidates against different endpoints
113 using different cancer cells [26, 27]. In the current preclinical setting of anticancer discovery,
114 HTS *in vitro* assays together with combinatorial chemistry have become a standard tool to
115 readily identify agents with clinical potential. *In vitro* assays have been widely applied in various
116 cancer preclinical studies and diverse platforms such as NCI60 [28], LINCS project led by NIH
117 [29], and anticancer drug sensitivity studies from both the Broad Institute [30] and the
118 Wellcome Trust Sanger Institute [31].

119 There are two major types of *in vitro* assay approaches for anticancer drug discovery –
120 phenotypic screening and target-based screening. Unlike the target-based approaches based on
121 engineered cloned genes either in cell-based or biochemical *in vitro* assays, phenotypic
122 screening assays have relatively straightforward endpoints for ameliorating the cancer
123 phenotype, which are exemplified by selectively killing cancer cells, eliminating cancer cell
124 proliferation or decreasing the cancer cell size [32]. There is some debate on which technology
125 contributes more to discovery of first-in-class drugs [33, 34]. Based on FDA approved drugs
126 statistics (1999 ~ 2008), phenotypic screening took more first-in-class drugs to the market than

127 target-based approaches. However, another much larger scale of studies based on from 1999 to
128 2013 drew an opposite conclusion that 78 of 113 FDA-approved first-in-class drugs are based on
129 target-based approaches. In the anticancer drug area, target-based approaches introduced
130 more anticancer drugs to the market [35], but both types of screening have their own values
131 and can lead to viable drugs [37, 41]. The target-based screening approach is hypothesis-driven,
132 in which cancer disease modeling and pathway analysis leads to a candidate protein or
133 proteins. Compounds that perturb or interfere with the candidate protein are considered as
134 lead compounds. The target-based approaches have had a lot of success, especially in kinase
135 inhibitors [36]. Between 1999 and 2013, 21 of 31 oncology new molecule entities (NMEs)
136 discovered by target-based approaches are kinase inhibitors [32]. However, the target
137 identification and validation for anticancer drug development is of great challenge. First,
138 validated anticancer drug targets are far more difficult to identify than we expected. Candidate
139 anticancer targets are initially identified from different biological based HTS efforts, which is
140 mainly hypothesis-driven. Therefore, further in-depth validation experiments are needed to
141 establish that the proposed candidate targets have desired therapeutic effects and low risk
142 [37]. There are fewer than 100 anticancer targets implicated in FDA-approved anticancer drugs,
143 which is still a small proportion compared to the 20,000 human genes that encode
144 approximately 500,000 proteins in the human genome [38, 39]. Furthermore, due to the limited
145 and incomplete knowledge of cancer-related proteins involved in specific human malignancies,
146 even drug candidates with high potency identified in the screening process may have little or no
147 value. For example, colorectal tumors harboring a KRAS mutation that activate the EGFR
148 protein signaling pathway fail to respond to EGFR inhibitors such as cetuximab (*Erbitux*) in
149 mutated KRAS-related colorectal patients [40]. Also, some cancer-related tumor-suppressor
150 genes such as RAS are not directly “druggable”, which creates another hurdle to apply target-
151 based screen approaches [41, 42]. For example, the GTPases were identified as the key
152 enzymes to activate RAS protein. Therefore, efforts were made to inhibit GTPases to control
153 RAS activation. However, the low molar affinity between small molecules and GTPases made
154 inhibiting GTPases untenable. Furthermore, RAS protein function is highly associated with the
155 inner face of the plasma membrane, further complicating controlling RAS activation, since small

156 molecules could not reach the RAS protein. [43]. Some advanced cell-based assay technologies
157 including 3D *in vitro* assay models [44], organ-on-a-chip systems [45-47], cellular imaging [48,
158 49], and iPSC stem cells [50] may improve the performance of target-based *in vitro* assay
159 performance. For instance, the multicellular co-culture system mimics the tumor
160 microenvironment by migrating tumor cells to adjunct microenvironment cell types such as
161 endothelial cells and fibroblasts, thereby modeling the complex pathological features of
162 different cancer types. This strategy has been applied for drug efficacy screening for breast
163 cancer [51].

164 Meanwhile, phenotypic based screening seems to be experiencing a resurgence in anticancer
165 drug discovery [52, 53]. Phenotypic *in vitro* screening is considered as a semi-empirical
166 approach that does not require knowledge of the underlying mode of action and molecular
167 mechanisms of the compounds being evaluated. Cancer phenotypes can be observed in cell
168 lines, and thus compounds that disrupt that phenotype may be viable drugs. In particular,
169 human primary cells, immortalized primary cells, and iSPCs have been widely applied to the
170 phenotypic screening assays, which has provided a lot of success in anticancer drug discovery
171 [34]. One example is carfilzomib, which is a selective proteasome inhibitor used to treat
172 multiple myeloma after the patients received prior therapies such as bortezomib and
173 lenalidomide. Proteasome inhibitors could induce apoptosis and inhibited tumor growth.
174 Carfilzomib could reversibly bind to the chymotrypsin-like (ChT-L) active sites in the 20S
175 proteasome, which potently control the cell growth and proliferation. The efficacy of
176 carfilzomib was originally discovered by using a cytotoxicity screen [54]. One difficulty of the
177 phenotypic screening approach is dosage optimation since there is no clear target for the
178 cancer types. Other challenges include optimising chemistry against an unknown target and
179 prediction of unwanted toxicities that may normally be elucidated from target distribution.

180 Besides considerations regarding the biological nature of cell-based assays for anticancer drug
181 discovery, the quality of HTS assays and how to interpret the results also play a role in better
182 harnessing the technology. One example is the inconsistency in two large drug response data
183 sets from the Cancer Cell line Encyclopedia (CCLE) [30] and the Genomics of Drug Sensitivity in

184 Cancer [31] based on cell-based HTS assays [55]. There are 15 common drugs characterized in
185 431 cancer cell lines between the two studies, which showed a substantial divergence in drug
186 response, although the gene expression similarity is well-established [55, 56]. There are a lot of
187 underlying reasons contributing to the divergence. The batch effect of fetal bovine serum used
188 in the different studies, the mathematical equation employed in curve-fitting of concentration-
189 response curves, and even the coating on plastic wells may be influential. Another potential
190 influence on this divergence is which measure, quantitative or qualitative, should be used as
191 the assay endpoint. For example, the method employed to measure the metabolic activity by
192 assessing levels of the energy transfer molecule ATP, could influence the assay's endpoint,
193 contributing to the observed divergence. Due to these concerns, *in vitro* assay results should
194 not be interpreted as a pure statistical measurement, but rather interpreted in the context of
195 the generated hypotheses that each drug was tested under. [57]. Undoubtedly, the
196 reproducibility of cell-based screening assays for anticancer drug development is of great
197 importance [58, 59]. Considering cell-based assays are plagued with the concerns of false
198 positive and false negatives [60], the statistical practices [61] and application domain of assays
199 [62] need to be standardized and defined [63]. Wassermann *et. al.* [64] revisited the screening
200 collection that never showed biological activity based on HTS techniques, and therefore
201 became defined as 'dark chemical matter' (DCM). It was found that some of the false negative
202 compounds based on HTS screening did show biological relevance under the quality control
203 assays such as prospective reporter-gene assay gene expression experiments. Therefore, critical
204 data quality control and wise design of experiments is a "must" to ensure reproducible and
205 reliable results generated from cell-based assays [65].

206 **Animal models**

207 Animal models are widely used to verify the biological relevance of the identified target for
208 tumor response, to predict the first-in-human (FIH) dose and maximum tolerated dose (MTD),
209 to determine the potency of anticancer drug exposure target, and to detect the qualified pre-
210 clinical prognosis, diagnosis and predictive biomarkers [66, 67]. The principle behind animal

211 models is that the physiological features of animals closely resemble humans in genetic,
212 epigenetic, and environmental factors, which is open to debate.

213 The lessons learned from animal models in anticancer drug development are mainly related to
214 how animal models could better resemble cancer pathophysiology in humans. The challenges in
215 extrapolation from animal studies to humans for anticancer drug development may be not only
216 attributed to the technical and biological transferability of the animal model itself but may also
217 involve the design, execution, and interpretation of the results from animal models [68]. Below
218 we explore lessons learned on how to improve the animal model performance such as animal
219 model application, and PK/PD model optimization (**Figure 3**).

220 *Application domain of animal models*

221 Various cancer animal models have been developed to mimic patient tumors, including human
222 cancer cell-line based xenograft models [69], patient-derived xenografts (PDXs) [70-72],
223 immune-competent models [73], and genetically engineered mice (GEM) [74]. The pros and
224 cons of different kinds of animal models for anticancer drug development have been intensively
225 discussed [66, 75-77]. The human cell-line based xenograft model was established by using the
226 mouse as an immune-deficient host for transplanted human cancer cell-line growth. The classic
227 example of human cancer cell-line based xenograft model is so called athymic 'nude
228 mouse'[69]. The transplanted human cancer cell-line model is easily tractable, controlled and
229 experimentally convenient. However, there are also some shortcomings of this cell-line based
230 xenograft models. First, the nude animal is immune-deficient, which does not resemble the
231 immune environment of human tumors. Therefore, the human cell-line based xenograft models
232 are not applicable for immune-related anticancer drug development. Second, because cell lines
233 adapt through the clonal selection process as they grow on plastic, they do not repeat the
234 genetic diversity seen in human tumors, nor do the cell lines reflect intratumoral heterogeneity.
235 Additionally, the human cancer cell line is typically extracted from early-stage cancer patients.
236 Finally, the subcutaneous location may not foster important tissue-specific stromal infiltration,
237 which means the model is a poor fit for soft tissue sarcomas with typical tumor growth. Due to

238 these limitations, some reports suggest combining the different human cell line types in
239 xenograft models may improve the performance, which has been successfully for ER+ and
240 triple-negative breast cancers[78]. The human PDX model, which directly implants the human
241 tumors into a mouse, has been widely applied in both academia and industry for anticancer
242 drug development [79, 80]. The PDXs suffer similar concerns as the human cancer cell-line
243 based xenografts regarding to lack of immune features and difficulty of tumor growth in
244 subcutaneous regions. However, the PDXs could better replicate the mutational heterogeneity
245 and reflect the intricacies of tumor subpopulations [81]. For example, some mutation-related
246 cancer subtypes such as mutated ESR1 related ER+ breast cancer could be identified only in the
247 PDX model but did not show any signal in cell-based xenograft [82]. One of the big concerns of
248 animal models is how to mimic the immune-comprised systems of cancer patients in the mice.
249 The immune-competent models and genetically engineered mice (GEM) successfully reproduce
250 the immune features and tumor interaction in animals by employing different bioengineering
251 techniques [83]. The immune-competent model is established by transplanting mouse cell line
252 and tumor tissues to the immune-competent host with immune cells and fibroblast
253 incorporated. The immune-competent models provide interactive immune system features and
254 mimic tumor microenvironment, thus more closely approximating human cancers. However,
255 the limited available cell lines for immune-component models coupled with rapid and
256 uncontrolled cell growth limit its wide application[66]. The GEM model aims to manipulate the
257 mouse genome to introduce the germline mutation or conditional mutations for different
258 tumor types. Especially with the rapid development of gene editing technology, the GEM model
259 has a promising future for cancer etiology, epigenomics and personalized cancer treatment
260 [74]. One promising example of application of the GEM model for anticancer development is
261 selumetinib (clinical Phase I/II/III), which is designed for multiple cancer types including triple-
262 negative breast cancer [84], non-squamous cell lung cancer [85], pancreatic cancer [86], and
263 neurofibroma [87, 88]. One of the indications is KRAS-mutant non-small cell lung cancer
264 (NSCLC). A co-clinical trial that combines preclinical and clinical models was employed to
265 observe the drug response (selumetinib and docetaxel) for NSCLC in humans and in genetically
266 engineered mice and found the selumetinib could significantly increase the efficacy of

267 docetaxel, a standard chemotherapy [85]. Meanwhile, there are also many cases of failure of
268 animal models in anticancer drug development (**Table 1**).

269 No single animal model will fit all purposes. For example, the cell-based xenografts and human
270 PDXs models are more suitable for tumor-cell-derived signal detection such as cell death and
271 proliferation but not fit for immune-related anticancer drug discovery. GEM models and
272 immune-component models may not be useful for intratumoral subclonal identification due to
273 limited types of mutations and technical hurdles for monitoring internal organs[89]. A
274 combination of animal models and cell based *in vitro* assays could provide more robust results.
275 Furthermore, some novel animal models such as the 3D organoids based cell-line xenografts
276 may also offer alternative means to further update and improve animal model
277 performances[90].

278 *PK/PD model optimization*

279 Anticancer drugs are considered as one of the most toxic drug classes in the therapeutic
280 spectrum [12, 91, 92]. Associated adverse drug reactions cover almost every organ system and
281 are known to cause multiple organ toxicities, which could be explained by the nature of
282 anticancer drugs which are intended to kill cells together with their tendency to off-target
283 promiscuity [93]. A major difficulty is the unexpected side effects observed in the clinical phase
284 that could not be detected in animal models, and *vice versa*. Dose is the key factor to balance
285 the efficacy and safety profiles for anticancer drugs [94-96]. Due to the anticipated toxicities,
286 Phase I clinical trials are often conducted in cancer patients under The International Council for
287 Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)S9 .
288 However, for some less toxic anticancer therapies such as targeted therapies, Phase 1 trials may
289 be conducted in volunteers under ICH M3. For these latter trials, one of the most important
290 tasks for animal models is to establish the maximum recommended starting dose (MRSD) for
291 clinical Phase I study for healthy human volunteers. The FDA has developed guidelines for the
292 industry such as "*Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial*
293 *Clinical Trials for Therapeutics in Adult Healthy Volunteers*". The conventional MRSD dose

294 prediction strategies are based on no observed adverse effect level (NOAEL) [97] and the
295 minimum anticipated biological effect level (MABEL) [98] approaches and have been widely
296 applied to first-in-human (FIH) dose estimation. Currently, the FIH dose is typically calculated by
297 using one tenth of the toxic dose in 10% of the animals (STD10), which is the dose that causes
298 severe toxicity in 10% of rodents [99]. Typically, at least two species are required in
299 toxicological studies: one rodent such as the rat or mouse) and one nonrodent such as the dog,
300 minipig or monkey.

301 PK/PD models play an increasingly important role in preclinical studies [100]. Since anticancer
302 drugs often have a very narrow therapeutic index (TI), a more precise PK/PD model is required
303 to estimate the FIH dose. Novel PK/PD models tends to combine diverse properties including
304 pharmacology (potency, selectivity), preclinical safety profiles (doses and exposure related to
305 toxicity), risk assessment (target and chemical assessment) and surrogate biomarkers such as
306 those related to clinical and toxicity endpoints into the same framework to better predict the
307 FIH dose [101-104]. One recent example added a pharmacogenomics dimension to the PK/PD
308 model to define equivalent PK/PD dosing regimens for different genetically distinct tumor
309 models [105]. Such a concept has been successfully used to define the FIH dose of epidermal
310 growth factor receptor (EGFR) inhibitors such as gefitinib for different EGFR mutation carrier
311 groups [106].

312 Preclinical models may perform well and effectively, but only when the context is well -defined
313 and data are interpreted with care. To reproduce successful cases and apply valuable
314 experience into anticancer drug preclinical practice, the comprehensive and critical re-
315 evaluation of cell-based and animal models is essential. Some of the large-scale consortium
316 efforts and available public datasets make it possible to conduct meaningful retrospective
317 analyses of quality control suitability of the disease context and the utility of preclinical
318 anticancer tools [107-109]. Furthermore, some alternative approaches such as the Phase 0
319 clinical trial may be a promising complementary tool in pre-clinical anticancer models [110-
320 112]. A phase 0 clinical trial is conducted prior to the conventional clinical phase I dose
321 escalation, tolerability assessment, and safety evaluation with limited human expose (usually

322 10-15 patients) and short period (typically with one week) and aims to optimize the PK/PD
323 features especially oral bioavailability and half-life of anticancer drugs.

324

325 **Divergence between Clinical Phase II and Phase III**

326 According to statistics of Clinical Development Success Rates between 2006-2015
327 (https://www.bio.org/sites/default/files/Clinical%20Development%20Success%20Rates%20200
328 6-2015%20-%20BIO,%20Biomedtracker,%20Amplion%202016.pdf), anticancer drug
329 development suffers a higher failure rate (75.4%) from clinical Phase II to Phase III when
330 compared to non-oncology drugs (65.7%). The FDA recently published a report entitled "*22*
331 *Case Studies Where Phase 2 and Phase 3 Trials Had Divergent Results*"
332 (http://www.fda.gov/aboutfda/reportsmanualsforms/reports/ucm535541.htm). Among the 22
333 cases, five drugs (5/22=22.7%) are oncological agents (see **Table 2**). The major reason for
334 anticancer drug failure from clinical Phase II to Phase III is lack of efficacy [113, 114].
335 Improvement of survival rate in patients is considered as the gold standard for anticancer drugs
336 in clinical trial. Clinical endpoints such as overall survival (OS), disease-free survival (DFS),
337 progress-free (PFS), time to progression (TTP) are also widely applied in cancer clinical studies.

338 One of the difficult lessons from the past few decades of anticancer drug development is that
339 positive results in Phase II do not guarantee a subsequent success in Phase III. This could be
340 because the limited patient population in Phase II trials may not accurately reflect the broader
341 patient population in Phase III trials. Furthermore, clinical endpoints in Phase II may be related
342 to controlling signs of disease over the short-term such as PFS which is easier to achieve than
343 the desired clinical endpoint for success in Phase III, which is lengthening lifespan . Thus, these
344 two endpoints may not correlate. In addition, the statistical measure in the smaller Phase II
345 population may suffer from over-fitting, in which the benefits ascribed to the drug treatment
346 are actually the result of random noise, and thus do not translate into larger populations.
347 Specifically, the statistical model may outperform within the context of Phase II but not within

348 the extended patient population in the clinical Phase III. Alternatively, there may be simple bias
349 which is less likely to occur in the larger patient population in Phase III.

350 Elimination of the divergence between Phase II ("therapeutic exploratory") and Phase III
351 ("therapeutic confirmatory") is the key to improving successful rates for anticancer drug
352 development. Patient recruitment in the late-stage clinical trials has been a great stumbling
353 block. Around 20% of cancer clinical trials were never finished due to insufficient patient
354 enrollment, which is largely attributed to uncertain benefit to the cancer patients participating
355 in the trials [115] n addition, more sensitive surrogate biomarkers are needed for use in the
356 clinical trials. Patient recruitment in a clinical trial is mainly based on the pathology and
357 morphology of diseases, which aims to collect homogeneous populations. However, patients
358 collected in Phase III are substantially genetically heterogeneous [116]. For instance, the
359 patients may carry different genetic mutations that are related to wholly different cancer
360 subtypes, and therefore the compound under evaluation may have widely varied effects on
361 these diverse tumors. With the advances in high-throughput "omics" techniques, it is possible
362 to collect more information on patients such as genetic background and epigenetic properties
363 to facilitate patient recruitment in Phase III.

364 The divergence among the population does not just exist in different clinical trial phases but
365 also manifests in the post-marketing stage. One example is bevacizumab (Avastin®).
366 Bevacizumab a vascular endothelial growth factor (VEGF) inhibitor, is the best-selling
367 anticancer drug in the world, which was approved to treat multiple cancers such as colon
368 cancer, lung cancer, and glioblastoma. In 2008, bevacizumab was approved by the FDA to treat
369 metastatic breast cancer. However, this approval was revoked by FDA due to hypertension and
370 kidney toxicity and poor progression-free survival profiles from post-marketing studies [117].
371 Another example is ponatinib, a BCR-ABL tyrosine kinase inhibitor (TKI). In clinical Phase II
372 studies of ponatinib, there were a total of 449 patients involved. Among the 449 patients, only
373 the 128 patients carrying the T351l mutations in BCR-ABL had a positive response to the
374 treatment. However, ponatinib was given fast-track approval by the FDA on the basis of these
375 Phase II results, and as such, was approved for chronic myeloid leukemia (CML) for the general

376 population in December 2012. Then, some published results the following year reported
377 incidents of severe cardiovascular toxicity in patients taking ponatinib, causing the FDA to
378 suspend approval of the drug. Just seven weeks later, the FDA provided guidance to
379 reintroduce ponatinib back to the U.S. market for a more specific patient group (T351I mutation
380 carriers) with CML [118]. These two examples highlight the divergence between the clinical
381 study and general population groups and its consequence to the anticancer drug approval
382 process, which also stimulates us to rethink the current clinical design of anticancer drug trials.
383 First, the current clinical endpoints for anticancer drugs are focused on the time-to-event type,
384 which creates a lot of problems when translated from one clinical phase to another due to both
385 unclear biological meaning and statistical measures. More effective biomarkers relevant to
386 cancer pathology and drug pharmacology are urgently needed to improve the translation from
387 one clinical trial to the next. Biomarkers that more accurately reflect the efficacy and clinical
388 benefits of anticancer treatment may improve performance of the compounds in clinical trials.
389 Examples of possible biomarkers include circulating tumor DNA, concentration of antigen KI67
390 in the serum level, and circulating tumor cell (CTC) counts [117]. Secondly, the clear endpoints
391 and desired target population should be fully taken into consideration in the design of clinical
392 trials and patient recruitments. Specifically, the genetic background of recruited patients could
393 be helpful to identify patients with specific genetic mutations most likely to benefit from the
394 study drug. With the decreased cost (less than $1,000) of sequencing techniques and advanced
395 PCR assays, it is more possible to implement genetic testing as part of clinical trials.

396 **Anticancer drug resistance**

397 One of the chief lessons that has emerged in the past two decades of anticancer drug
398 development is that the promise of targeted therapy is tempered by the realities of drug
399 resistance. Cancer drug resistance, in which the tumor cells are either inherently unresponsive
400 to the treatment drug or develop changes that allow them to tolerate the drug, is one of the
401 biggest challenges in anticancer drug development. Some known mechanisms that promote or
402 induce anticancer drug resistance include drug transport and metabolism such as drug efflux
403 and drug activation/inactivation, drug target alterations, DNA damage repair and downstream

404 resistance mechanisms such as deregulation of apoptosis and autophagy [119]. These
405 mechanisms are divided into two categories: intrinsic and acquired. Intrinsic resistance means
406 the resistant is pre-existing in the tumor cells before the chemotherapy. Acquired resistance
407 occurs in the cancer development process, which can involve sub-cloning of tumor somatic
408 mutations, increased target expression level and recruitment of alternative compensatory
409 signaling pathways [120]. Moreover, molecular and genetic heterogeneity present in tumors
410 contributes substantially to drug resistance [121].

411 Although diverse underlying mechanisms of anticancer drug resistance have been deciphered in
412 the past two decades, we still have a long road ahead before we have sufficient knowledge to
413 overcome this issue. [119]. Often tumors display multiple drug resistance (MDR), which is one
414 of the major reasons for ineffectiveness and toxicity of chemotherapeutic agents [122]. The
415 ATP-binding cassette (ABC) transporter family was identified as one of the causal factors for
416 MDR. There are a total of 49 ABC transporters. However, very few proteins such as MDR1,
417 MPR1, and BCRP have has been studied and identified in relation to MDR [123]. Initial efforts to
418 develop ABC transporter inhibitors such as MDR1 inhibitors to overcome tumor resistance have
419 yielded disappointing clinical outcomes. The first generation of MDR inhibitors had low affinity
420 for ABC transporters, and increased dosing caused unexpected side effects [124]. The second
421 and third generation MDR inhibitors had improved pharmacological profiles with higher affinity
422 to ABC transporter. However, the clinical effectiveness is still suboptimal. For instance, the
423 MDR1 inhibitor tariquidar was proposed as an adjuvant against multidrug resistance in late-
424 stage breast cancer. However, the clinical trial (phase II) showed no benefit for patients'
425 survival[125]. The possible reason may be the functional redundancy within the ABC
426 transporter family or that other contributors, beyond ABC transporters, affect tumor resistance.
427 Some preclinical cell-based HTS screening panels has been developed for ABC transporter
428 screening, which could be important reference information in monitoring potential MDR [126].
429 Furthermore, rational drug combinations have been proposed to conquer MDR by targeting
430 multiple components of the cancer process to improve the efficacy and overcome tumor
431 resistance [127]. Research increasingly indicates that drug combinations that target multiple

432 pathways are more effective than targeting multiple targets within the same cancer-related
433 pathway [119]. Tumors evolve over time in terms of their epigenetics, genetics and gene
434 expression levels, which causes tumor initiation, metastasis, and drug resistance. Mutations
435 that arise in the early stage tumors could further evolve into very different mutation types,
436 which may cause the tumor to adapt and develop resistance to treatment. One example of
437 evolving mutations is provided by gefitinib, which is an epidermal growth factor receptor
438 (EGFR) inhibitor designed for non-small-cell lung cancer (NSCLC) treatment. Gefitinib is effective
439 in patients with specific activating mutations in EGFR such as L858R in exon 21 but these
440 benefits often last only for the first year of treatment. However, the evolving tumor acquires a
441 new gatekeeper mutation named EGFR-T790M to maintain the genetic information and control
442 the tumor growth, which cause 50% of patients to experience drug resistance and ultimately
443 treatment failure [128]. In some cases, researchers have designed second-generation drugs that
444 overcome the initial resistance. One example is the BCR-ABL1 oncogenic kinase inhibitors for
445 chronic myeloid leukemia (CML). The first BCR-ABL1 inhibitor was effective but patients
446 relapsed due to sub-cloning of the T315I mutation of BCR-ABL1. Drug makers developed the
447 second generation of BCR-ABL1 inhibitors such as dasatinib and bosutinib against the T351I
448 mutation in BCR-ABL1 [129].

449 With the wide spectrum of cancer drug resistance mechanisms, it seems unlikely that the
450 dream of a "magic bullet" to cure cancer will ever be realized [119]. However, we should not
451 lose sight of significant progress being made as anticancer drugs have become more precise
452 and have prolonged and improved patients' lives. With the assistance of modern omics
453 techniques, we are experiencing a substantial increase in our ability to identify the molecular
454 mechanisms for cancer drug resistance. Thus, the cumulative experience of cancer drug
455 resistance research, from conventional chemotherapy to target-based therapies, can serve as
456 the foundation to drive further research and to increase the number and effectiveness of
457 anticancer drugs.

458 New trends for anticancer drug development

As novel technology is increasingly applied to the challenge of cancer, new opportunities are emerging to innovate in anticancer drug development. Here is a glimpse at some of the great strides including precision medicine, cancer stem cells, and drug repositioning (more in **Figure 4**).

Precision medicine

Precision medicine is an approach to integrate molecular and clinical information to better understand of disease by using novel genomics techniques such as next generation sequencing [18, 130]. Precision medicine aims to utilize the unique genetic profiles of patients to look for better treatment solution, which provides the right drug, the right dose to the right patients with reduced safety concern.

Targeted cancer therapy as an important practice of precision medicine is considered as an indispensable components of current anticancer drugs development [131]. Unlike the conventional chemotherapy, targeted cancer therapy works on the specific target in cancer-related molecular pathways to treat cancer. Targeted cancer agents are broadly divided into small molecules and monoclonal antibodies. The small molecule based targeted cancer agent is able to interact with the target inside the cell by penetrating the cell membrane, and monoclonal antibodies are designed to target specific antigen on the cell surface. For example, trastuzumab as a monoclonal antibody is designed to treat HER2 related breast cancer, which is only beneficial to the patients with HER2 protein overexpressed [132]. The precision medicine provides more deep resolution of genetic feature of cancer patients, which makes the patients with different genetic mutation as a group to receive the specific treatment option possible. The successful examples include imatinib for patients with chronic myeloid leukemia carrying a BCR-ABL mutation [133] and vemurafenib for those with melanoma or thyroid cancer who have the BRAF V600E variant [134]. The implementation of precision medicine requires the integration of molecular diagnosis into the anticancer drug discovery process [135]. Currently, there are approximately 35% (71/203) established pharmacogenomics biomarkers for approved anticancer drugs and incorporated into FDA-approved drug labeling .

486 The qualified biomarker or "fit-for-purpose" biomarker is the key to precision medicine practice
487 [136]. Rich resources on genetic variants and their relationship in human cancers are available
488 [137, 138]; however, understanding of how to leverage these findings into clinical practice
489 (from the relationship, correlation to translation) is still suboptimal. Furthermore, there are
490 some concerns over how many patients could actually benefit from precision medicine [139,
491 140]. One disappointing report on personalized cancer treatment based on genetic biomarkers
492 found only 30% patients had a positive response to personalized cancer treatment strategy and
493 this amounted to an average two-month improvement of progression-free survival [141]. As
494 highlighted recently, in 'precision medicine' the word 'precision' is being used in a colloquial
495 sense, to mean both 'accurate' and 'precise'. Precision implies a high degree of certainty of an
496 outcome but in fact, the opposite will probably result. The new tools for tailoring treatment will
497 demand a greater tolerance of uncertainty, a greater ability to interpret 'omics' data and a
498 greater facility for calculating and interpreting probabilities than we have been used to as
499 physicians and patients [142, 143]. Furthermore, although the price of next generation
500 sequencing for diagnosis is continually decreased, the expanse for development personalized
501 medicine based on individual genetic characteristics is still huge. Therefore, more efforts should
502 be encouraged to standardize precision medicine practice in both clinical translation and the
503 regulatory setting [144, 145].

504 **Cancer stem cells**

505 The discovery of cancer stem cells (CSCs) in the late 1990s triggered intense research efforts
506 into this specialized subpopulation of tumor cells. CSCs, also referred to as tumor-initiating cells
507 (TICs) can self-renew and drive tumorigenesis [146, 147]. CSCs play an important role in cancer
508 initiation [148, 149], maintenance [150, 151], metastasis [152] and recurrence[153-155].
509 Therefore, a lot of efforts have been made to decipher CSC function in cancer pathogenesis,
510 and to apply these findings in anticancer drug development [156].

511 To date, CSCs have been discovered in multiple types of solid tumors such as breast cancer
512 [157], lung cancer [158], and brain cancer [159]. Some targeting cellular surface markers

513 including *CD133* [160], *CD90* [161], *CD33* [162] and *PKA* [163], key pathways such as *Norch*,
514 *Hedgehog*, *Wnt*, and *NF-κB* signaling pathways [164], and transporters including ATP-binding
515 cassette (ABC) transporters [165] have been detected in CSCs. Studies have sought ways to
516 specifically target CSCs. Fang *et. al.* [166] performed HTS screening of small molecules and
517 found LF3 (a 4-thioureido-benzenesulfonamide derivative) could effectively block the self-
518 renewal of cancer stem cells and suppresses tumorigenesis. The finding was also verified by
519 using a mouse xenograft model of colon cancer. Masuda *et. al.* [167] found that small-molecule
520 Traf2- and Nck-interacting kinase (TNIK) inhibitor, NCB-0846 could downregulate Wnt/β-catenin
521 signaling by using $Tnik^{-/-}/Apc^{min/+}$ mutant mice, which is essential to maintain the function of
522 CSCs.

523 Translation of CSC research findings into anticancer drug development is still in the early stages
524 [168]. The underlying mechanisms of how CSCs contribute to cancer progression are still not
525 fully uncovered, and so efforts continue to unravel the biology [169-171]. However, CSCs
526 remain a promising tool in anticancer drug development. Novel strategies such as
527 nanomedicine targeting the CSC microenvironment are also being explored [172, 173].

528 **Drug repositioning**

529 Drug repositioning, an approach of finding new uses for existing drugs, has been attracting a lot
530 of attention [22, 174]. By integrating different biological, chemical and genomics data profiles,
531 drug repositioning can provide a rapid method to verify hypotheses and generate candidates
532 for clinical validation. With the successful clinical application of non-cancer drugs for cancer
533 treatment, drug repositioning becomes a powerful tool for anticancer development.
534 Considering cancer often involves multiple pathologies [175], drug repositioning for
535 combination therapy may be a promising direction [176].

536 Various drug repositioning approaches have been developed and could be potentially applied
537 to anticancer drug development with initial evidence coming from preclinical models or
538 controlled population studies (**Table 3**). The classic story is thalidomide, which was first
539 marketed in 1957 in West Germany as a sedative and hypnotic. Afterward, it was also used

540 against nausea or alleviating morning sickness in pregnant women. However, severe adverse
541 reactions characterized by birth defects occurred and 60% of affected children died. Later,
542 researchers found thalidomide could inhibit NF-ƙB and STAT3, and it was approved by the FDA
543 for treating multiple myeloma in 2006 Another example is metformin. Metformin, as a first-line
544 drug for type II diabetes, has been demonstrated to be an alternative therapy for multiple
545 cancers with both chemopreventive and chemotherapeutic functions by single or combination
546 therapy with other drugs [177-179]. The cancer prevention and anticancer activity of metformin
547 have been demonstrated in cell-based assays[180, 181], animal models [181, 182] and
548 controlled population studies [183]. Furthermore, aspirin as a nonsteroidal anti-inflammatory
549 drug (NSAID) has been reported to reduce cancer risk with regular intake. Currently, the world-
550 largest clinical Phase III trial is underway in the UK to evaluate aspirin for its potential
551 effectiveness to treat cancers such as breast, colorectal, and prostate [184].

552 The increasing interest in drug repositioning for anticancer treatment development is mainly
553 driven by the desire to use discontinued drugs and further exploit existing drugs with known
554 PK/PD properties and safety profiles [176]. Some promising directions for anticancer
555 repositioning include treating cancer by targeting the microenvironment, triggering immune
556 systems by approved drugs [185]. Brian *et. al.* [185] mapped 1309 drugs onto 221 immune cell
557 types based on their transcriptomic signature and predicted ~70,000 interactions. In addition,
558 the authors experimentally validated the influence of one candidate drug (clioquinol) on
559 neutrophil migration from the bone marrow to the blood in 6- to 12-week-old female C57Bl/c
560 mice to investigate how the drug perturbs the immune systems. The proposed methodology
561 may be useful for immune-related anticancer drug candidate profiling. However, attention
562 should be paid to the complex pathological and etiological features of cancers, which are very
563 different from other common diseases. For example, cancer patients are a vulnerable
564 population and a drug that does not have safety issues in healthier patients might trigger
565 problems for them, especially if used in novel combinations [186]. Furthermore, the rationale
566 behind non-cancer drugs treating cancer is that off-target effects driven by the
567 polypharmacology of non-cancer drugs could be beneficial to the cancer patents' survival. Since

568 the known PK/PD properties of non-cancer drugs are derived from data in the original
569 indication, it is not guaranteed that the PK/PD features are still the same. Accordingly, the
570 safety profiles should be also evaluated.

571 Concluding Remarks

572 By revisiting the anticancer drug development in the past two decades, we observe that a lot of
573 encouraging progress has been made to improve cancer patients' survival and quality of life.
574 Meanwhile, there are still a lot of hurdles and unsolved difficulties in anticancer drug
575 development (see the **Outstanding Questions**). Furthermore, anticancer drugs tend to
576 command extremely high prices due to unmet and urgent needs of the market and patients
577 [187]. We have highlighted here some successes from the past twenty years, along with the
578 challenges posed by translational from preclinical to clinical trials, from a small population to
579 the larger population, and limited qualified biomarkers in the anticancer drug paradigm. All
580 three of these issues draw attention to the need to reevaluate our current anticancer drug
581 development tools and redefine clinical context for their implementation. With the advantage
582 of biology, genetic engineering, and emerging techniques, more and more novel concepts such
583 as precision oncology and animal models such as PDXs have been successfully applied to drive
584 innovation in the anticancer drug discovery pipeline. However, utilization to truly harness these
585 advances to facilitate and accelerate anticancer development is still suboptimal. Some
586 uncertainties still exist with novel techniques, providing a barrier to robust and reliable results.
587 It is suggested that more perspective-retrospective studies should be conducted to build the
588 standards and guidance for application of novel anticancer development tools with
589 multidisciplinary efforts from regulatory agencies, drugmakers, and academic researchers. We
590 are delighted that a lot of activities have been advocated and promoted such as Cancer
591 Moonshot [188], Patient-Reported Outcomes (PROs) [189, 190], FDA Biomarker Qualification
592 Program , and PrecisionFDA , which build the communication bridges among the patients, drug-
593 makers and regulatory agencies to move this field forward.

Anticancer drug development covers a wide spectrum of multidisciplinary fields. Some points not touched on and covered in depth here also hold promise in anticancer drug development. For example, genetic elements such as miRNAs also provide a new avenue for looking for cancer treatment options [191]. In addition, one of the gene therapies approach aims to add new genes to a patient's cells to replace missing or malfunctioning genes [192, 193], which may play an important role in future cancer treatment development with precise gene editing technologies such as CRISPR/Cas9 gene editing now available [194, 195]. Furthermore, cancer-derived induced pluripotent stem cells (iPSCs) also provide a tremendous opportunity to model the effects of the cancer genome back to animal models for anticancer drug discovery [50, 196, 197].

Anticancer drug development has shifted from conventional cytotoxics agents to targeted-based therapy and immunotherapy in the past two decades. Whether the new concepts and models truly fit within the established anticancer drug development paradigm is still an open question. A rethink of the existing anticancer drug discovery pipeline could refresh our minds to define pitfalls and further improve successful rates. Furthermore, cancer drug development is a collaborative activity that requires drug makers, researchers, patients and regulatory agencies to form a cohesive strategy to accelerate and improve drug development to improve the life quality of cancer patients.

Resources

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) S9: https://www.fda.gov/downloads/Drugs/Guidances/ucm085389.pdf).

NCI cancer-related clinical trials: https://www.cancer.gov/about-cancer/treatment/clinical-trials/advanced-search

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) M3: https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292340.pdf

621 Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for
622 Therapeutics in Adult Healthy Volunteers:
623 http://www.fda.gov/downloads/drugs/guidances/ucm078932.pdf

624 Table of Pharmacogenomic Biomarkers in Drug Labeling:
625 https://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.ht
626 m

627 FDA Biomarker Qualification Program:
628 https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificatio
629 nProgram/BiomarkerQualificationProgram/default.htm)

630 PrecisionFDA: https://precision.fda.gov/

631 Seed and soil hypothesis:

632 http://www.nature.com/milestones/milecancer/full/milecancer01.html

633 NCI Cancer Statistics: https://www.cancer.gov/about-cancer/understanding/statistics

634 NCI drug repository: https://www.cancer.gov/about-cancer/treatment/drugs

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1037

1038 **Table 1** Examples of Divergency in anticancer drug candidates between Phase II-III*

Drug names	Sponsor	Therapeutic target	Indication	Notes
Brivanib	Bristol-Myers Squibb	VEGFR and fibroblast growth factor receptors (FGFR)	hepatocellular cancer	Lack of efficiency. Brivanib failed to improve overall survival of patients compared to approved drug (i.e. sorafenib) and also demonstrated identified unexpected side effects.
Iniparib	Sanofi	Poly(adenosine diphosphate–ribose) polymerase 1 (PARP1)	Triple negative breast cancer	Lack of efficiency. It was demonstrated that Inspired with standard chemotherapy regimen (gemcitabine and carboplatin) could not improve survival
MAGE-A3 vaccine	GlaxoSmithKline	Antigen for immune responses	non-small cell lung cancer (NSCLC)	Lack of efficiency. The clinical benefit could be proved when compared to a placebo
Velimogene A liplasmid (Allovectin-7)	Vical	Antigen for cytotoxic T-cell and innate immune responses	metastatic melanoma	Lack of efficiency. Allovectin-7 reduced tumor size significantly fewer patients than another two market drugs (i.e. dacarbazine and temozolomide) for late-stage melanoma patients.
Figitumumab	Pfizer	insulin-like growth factor-1 receptor (IGF-1R)	non-small cell lung cancer (NSCLC)	Figitumumab with the standard regimen (paclitaxel and carboplatin) fails to improve the survival. Furthermore, severe adverse events (SAEs) such as pneumonia, dehydration and even death were observed

1039 ***** The data is from "*22 Case Studies Where Phase 2 and Phase 3 Trials Had Divergent Results*" and curated from

1040 https://www.fda.gov/aboutfda/reportsmanualsforms/reports/ucm535541.htm

Table 2 Failed and on-going examples of animal models in anticancer drug development

Animal model	Tumor type	Involved drug candidates	Notes	References
Failed examples				
Cynomolgus and rhesus monkeys	B-cell chronic lymphocytic leukemia (B-CLL)	TGN1412 (agonistic anti-CD28 antibody)	Severe inflammatory reactions to immune system in Phase I	[198, 199]
Mouse medulloblastoma model	Malignant solid brain tumor (medulloblastoma)/ Pancreatic Cancer	Saridegib (Hedgehog pathway antagonist)	Lack of efficiency when compared to placebo in clinical phase II	[200, 201]
Mouse-derived portion of the scFv on the CAR T cell	Acute lymphoblastic leukemia (ALL) as well as relapsed or refractory (r/r) chronic lymphocytic leukemia and non-Hodgkin lymphoma (NHL)	JCAR014 (a chimeric antigen receptor (CAR) T-cell receptor, targeting CD22)	Patients Death due to in Phase I dose-escalation trial	[202]

Table 3 Examples of drug repositioning for cancer therapy

Drug	Original indication	Suggested cancer mechanism	Models	Notes	References
pioglitazone	Type 2 diabetes	Multiple types		Pioglitazone could stabilize the elevated expression of the iron–sulfur (Fe-S) protein nutrient-deprivation autophagy factor-1 (NAF-1), which is a key factor to cancer cell progression.	[203]
Flavopiridol	Under clinical development of acute myeloid leukemia	Glioblastoma	Human glioblastoma cell lines	Flavopiridol is a synthetic flavonoid that inhibits a wide range of Cyclin-dependent kinase, that has demonstrated to inactivate glycogen phosphorylase, decreasing glucose availability for glycolysis. It is suggested flavopiridol could combine with anti-proliferative agents to treat glioblastoma.	[204]
rapamycin	lymphangioleiomyomatosis	pancreatic cancer	Genetically engineered	Targeted anti-mTOR therapies may offer clinical benefit in subsets of human	[205]

			mouse models	pancreatic ductal adenocarcinoma (PDAC) selected based on genotype	
diflunisal	Anti-inflammatory drug	leukemia	leukemia cell lines and mouse model	Diflunisal can suppress the growth of p300-dependent leukemia cell lines expressing AML1-ETO fusion protein in vitro and in vivo	[206]
chloroquine	antimalarial drug	Multiple cancers	normal cells in mice and cancer patients	chloroquine (CQ), is a robust inducer of Par-4 secretion from normal cells in mice and cancer patients in a clinical trial. CQ-inducible Par-4 secretion triggers paracrine apoptosis of cancer cells and inhibits metastatic tumor growth.	[207]
JQ1 in combination with romidepsin	romidepsin is used to cutaneous T-cell lymphoma (CTCL) and other peripheral T-cell lymphomas (PTCLs)	Type II testicular germ cell cancers (TGCT)	TGCT cell lines and Embryonal carcinoma (EC) xenografted mice models	JQ1 in combination with romidepsin could reduce tumor size, proliferation rate, and angiogenesis	[208]

metformin	Type 2 diabetes	Multiple types	Cancer cell line and mouse/rat models	Metformin can inhibit mTORC1 pathway, which plays a pivotal role in metabolism, growth, and proliferation of cancer cell.	[177, 178, 180, 181, 183]

Figure Captions

Figure 1 **Milestones in anticancer drug development in the past two decades**

Figure 2 **The correlation between number of approved drugs and (A) percentage of survival in five years (2006~2012); (B) estimated newly added cases for each cancer type**: the statistics for each cancer type were based on the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute (https://seer.cancer.gov/statfacts/)

Figure 3 **The key factors for translation of animal results to humans in anticancer drug development:** species selection, applicability of animal models, toxicity profiles, and PK/PD model optimization. FIH dose = first-in-human dose; MTD = maximum tolerated dose

Figure 4 **New trends in anticancer drug development**

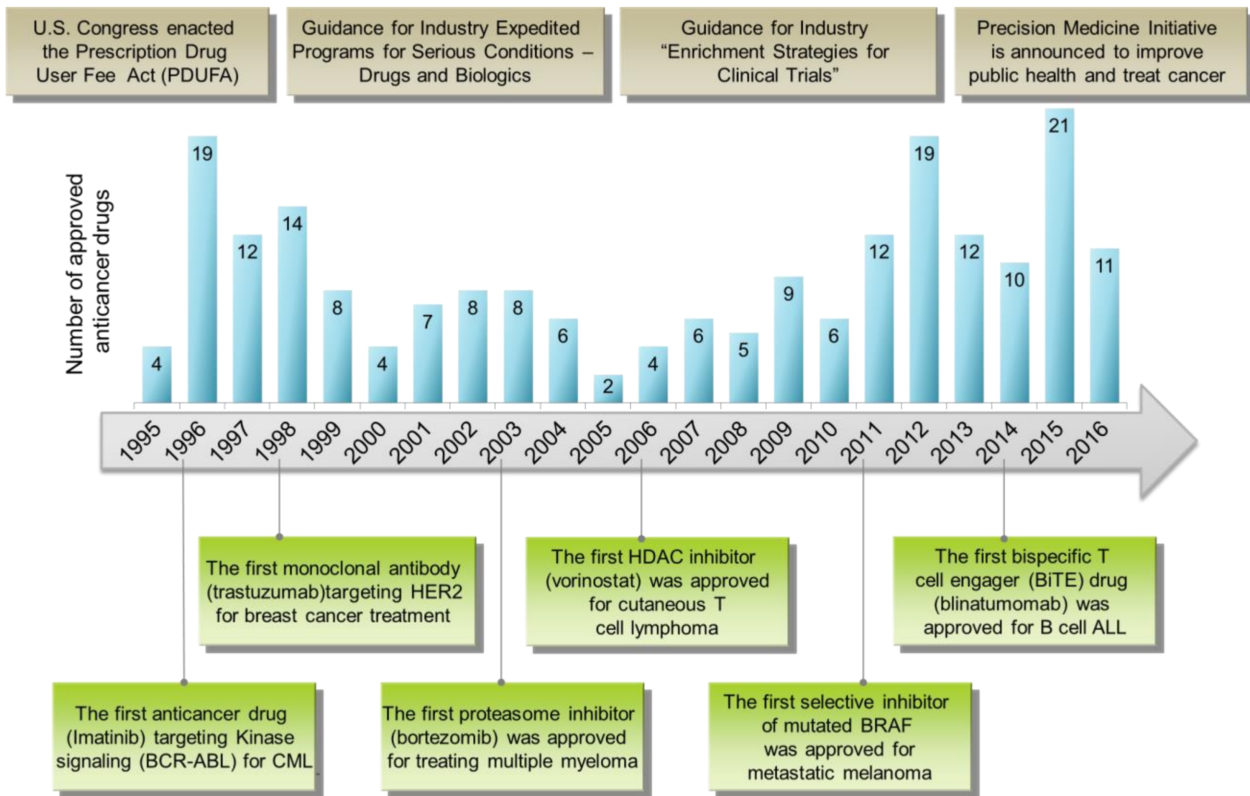

Figure 1

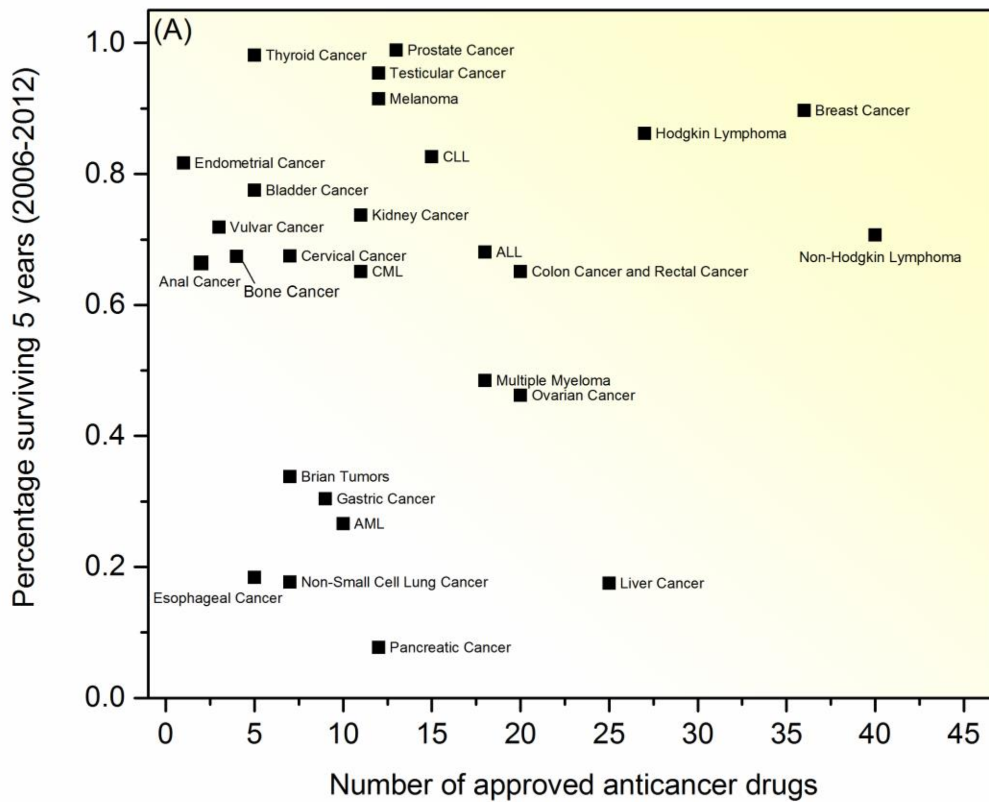

Figure 2(A)

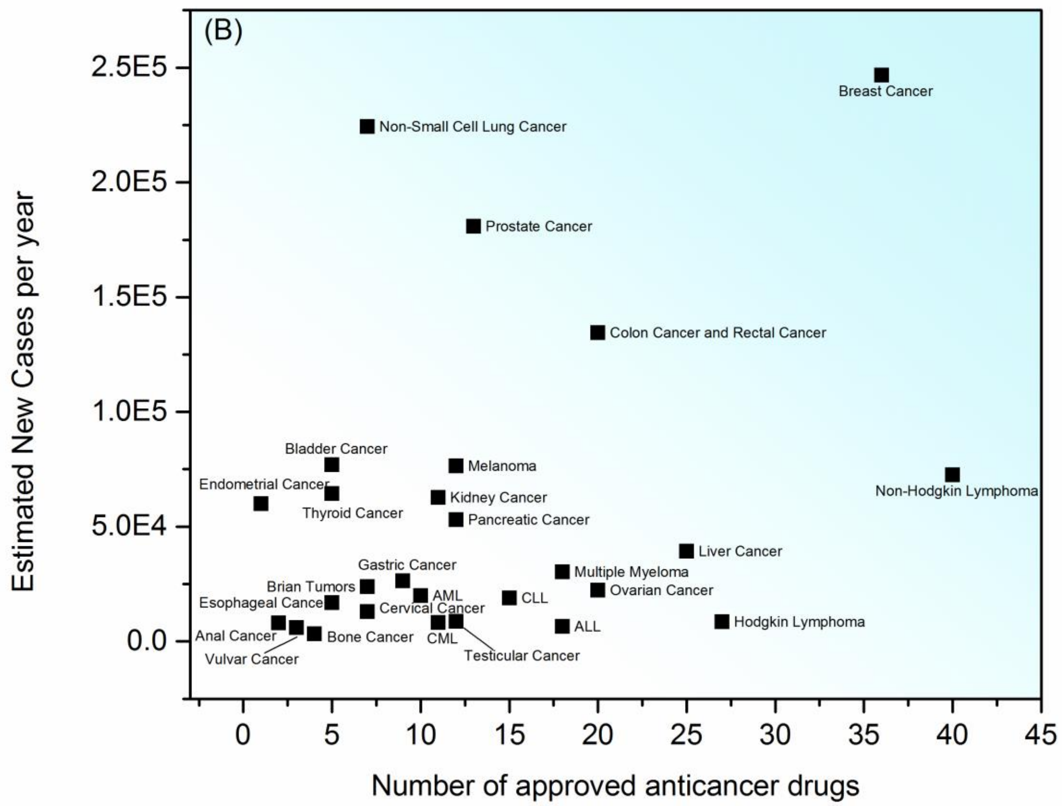

Figure 2(B)

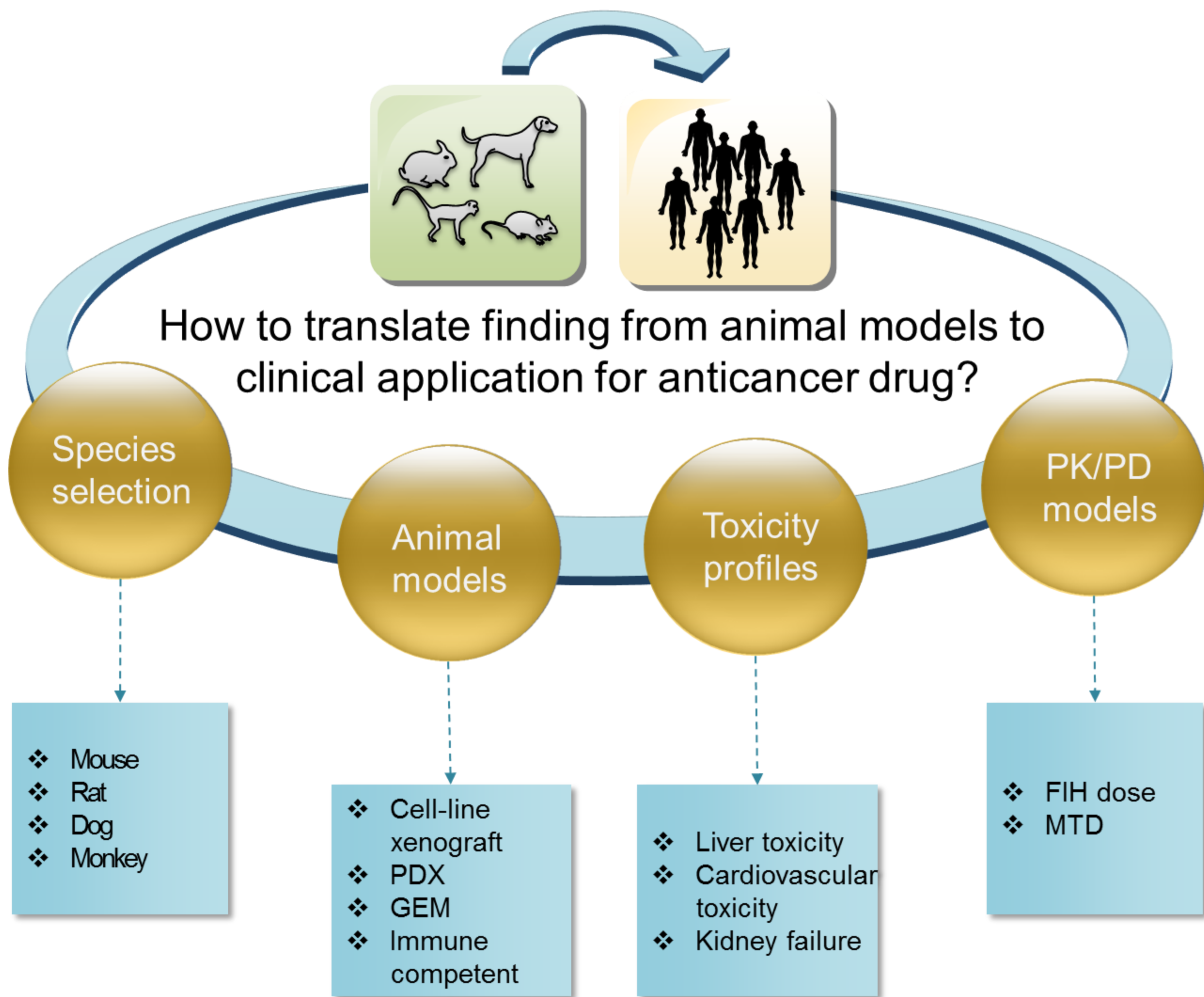

Figure 3

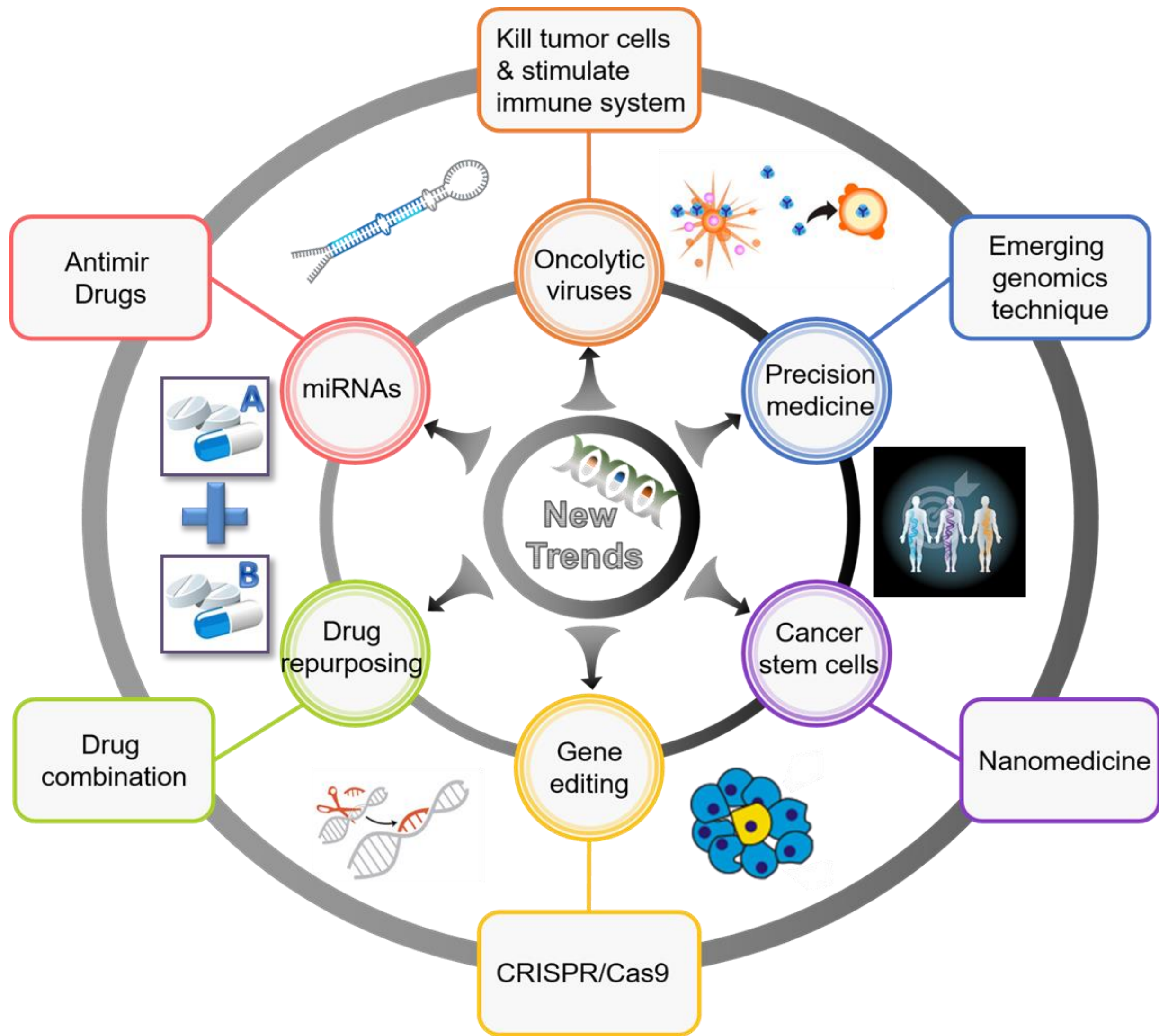

Figure 4