Chapter

What Are the New Challenges of the Current Cancer Biomarkers?

Jie Chen, Liqiong Yang, Yuxi Duan, Tinging Pu, Sha Zheng, Fangfang Liu, Kun Huang, Greg Mirt and Fan Xu

Abstract

Biomarkers are emerging research filed in the past decade. Even though numerous biomarkers were reported, the efficiency of cancer therapy remains low. So the question emerges as to how much can we trust the current biomarkers on cancer therapy? In this upcoming chapter, we would like to illustrate the outcomes of classical cancer therapies on advanced pancreatic cancer disclosed successful, neutral and failed in clinical trials. The analysis will be carried on the perspective interdisciplinary on the biomarkers including anatomy, physiology, pharmacology, biochemistry, history path and development of pharmacy. Particular in-depth insight may open a window for new researches and lighting the therapies.

Keywords: advanced pancreatic cancer, biomarker, clinical trials

1. Introduction

Advance pancreatic cancer (APC) is a highly lethal tumor. Most patients with APC remain asymptomatic until the disease reaches an advanced stage [1]. The incidence rate was 5.5 for men and 4.0 for women per 100,000 people. The mortality rate was 5.1 for men and 3.8 for women per 100,000 people around the world, according to data, from 2018 [2]. For the incidence rate, Asia is at 48.4%, Europe at 23.4%, and the Americas is at 21.0%. As for the mortality rate, Asia is at 57.3%, Europe at 20.3%, and the Americas is at 14.3% [3].

Our previous study disclosed that there were more than 19 chemotherapy regimens combinations in clinical practice available [4]. The advantages and disadvantages of each therapy regimens are clear. Briefly, to lengthen the overall survival and to reduce the treatment-related toxicity we must consider the outclass selection. There are more than 14 treatment-related toxicities in gastrointestinal, constitutional, skin, hepatotoxicity, infection, vascular, neuropathy, mental, pain, renal, electrolytes and pulmonary of human body in current dominant chemotherapy regimens. To broaden the balance requires expertise and professional medical training based on evidence.

2. Long period run in research and development in pharmacy

The development of drugs is based on the determination of new therapeutic targets, the pharmacological receptors. This concept was first proposed by Paul

Ehrlich in 1908 [5]. Normal cells replicate their DNA with great accuracy, but cancer has a large number of mutations that show up in cancer cells that make them pharmacological targets [6]. From the initial concept of molecular targets, drug targets were discovered and validated, which successful translated the most drugs into practice [7].

Excellent and reliable targets identification and validation can increase the credibility of the relationship between intentions and diseases, this may strength the effectively of drugs. Drugs are usually developed only when specific drug target for the action of these drugs are analyzed and examined. Sufficient potential targets have been discovered rapidly for the drug discovery process.

Numerous data including identified gene and drug discovery cycles have been generated exponentially. This may forge the difficultness in decision making and becomes more and more difficult for drug R&D. Thanks to rapid bioinformatic discoveries, more and more biopharmaceutical targets can be identified and analyzed [8].

Validation from cross-species a bioeffect is performed after the drug target is determined and verified. Rodents and non-human primates provide appropriate animal models for screening and evaluation of a new drug. Most of current cancer in vivo experiments use rodent experimental animals such as mice and rats. Because they are small, rapid reproduction, clear genetic background and mature genetic modification technology can be done. However, due to the distant relationship between rodents and humans, many of the results obtained from rodent models cannot be reproduced in humans. Moreover, non-human primates are highly similar to humans in terms of genetic evolution, immunity, physiology and metabolism. They are theoretically more suitable for cancer researches [9].

Clinical trials are the best channel to tie up pharmaceutical targets to reliable drugs. The goal is to determine whether a candidate drug is safe and effective. There are four phases in clinical trials. More specific biomarker studies are based on data from prospective studies [10]. In the study of cancer biomarkers, retrospective studies and prospective studies help to identify potential biomarkers, which may be validated in the future studies, however, the reliability of evidence remains controversial.

3. Can we trust the current biomarkers of cancer?

Unfortunately, the overall survival of APC patients has not revised assuredly. There are too many choices in clinical practice and evidence-based medicine is a permanent challenge. Which of the modern biomarkers is reliable? Are we ever going to detect precise pharmaceutical targets on APC [11]?

4. Validation method

In order to clarify this question, we collected the raw data source (http://clinicaltrials.gov) and searched all the drug treatments on APC. We refined all the data which had results and were published. Briefly, a total of 2726 recordings were found since May 2019. Hundred and fifteen recordings which finished clinical trials, further we ruled out irrelevant 32 recordings and 56 unclear results. Finally, 27 recordings kept comprising the following three tables. Raw data are free, please follow the link 10.6084/m9.figshare.8275190.

5. What can data tell us?

Total, there are 18 biomarkers used among these results. For details please see **Table 1**. The results from 27 clinical trials could be divided into three categories (a) rank of the effectiveness (b) rank of intervention and (c) quality of life improved.

5.1 Estimation of clinical trials with success outcome on advanced pancreatic cancer

Total, we found 10 publications where the author declares the successful outcome on the treatment of APC from 2011 to 2019, **Table 2**. These clinical trials recruited 1080 patients with APC (611 male and 469 female). The average overall survival month is 11.62 and progress-free survival month is 10.79. Briefly, Lutz et al. tests the GVAX pancreatic cancer vaccine via GPI biomarkers The OS and PFS approach got the highest point, 24.8 and 17.3, respectively Similarly, Phan et al. tested the pazopanib hydrochloride via VEGF biomarkers. This approach had a higher OS and PFS points. Survival months are 25 and 14.4. Furthermore, Hong et al. disclosed the capecitabine may put the OS and PFS to 17.3 and 10.4 survival months; the remain studies presented similar results, the OS and PFS were lower than 10 months.

Biomarkers	Abbreviation
Vascular endothelial growth factor*	VEGF
Thymidine phosphorylase	TP
Epidermal growth factor receptor*	EGFR
Tumor necrosis factor α	TNF-α
Topoisomerase I inhibitor	TIH
Sonic hedgehog	SHH
Severe hypoxia intracellular reductases	SHIR
Secreted protein acid rich in cysteine	SPARC
Platelet-derived growth factor	PDGF
MEK1/2-dependent effector proteins	ERK1/2
Kirsten rat sarcoma viral oncogene homolog*	KRAS
Interleukin 6/interleukin 8	IL-6/IL-8
Heat shock protein 27	Hsp27
Glycosyl-phosphatidylinositol	GPI
Double-strand breaks in DNA	—
Checkpoint kinase 1	CHK1
Microtubule-associated protein light chain 3- II	LC3- II
Circulating free DNA	cfDNA
Dihydropyrimidine dehydrogenase	DPD

NCCN Recommend: National Comprehensive Cancer Network.

Table 1.
Potential biomarkers used in advanced pancreatic cancer.

Year	Author	N	M	F	Drug	OS	PFS	Biomarkers
2011	Hill [12]	21	8	13	Capecitabine; docetaxel;	7.4	5.8	TP
					gemcitabine			
2011	Lutz [13]	60	37	23	GVAX pancreatic cancer	24.8	17.3	GPI
					Vaccine			
2011	Raymond [14]	86	42	44	Sunitinib	9	30	VEGF
		85	40	45	Sunitinib	21	51	
2013	Hosein [15]	19	9	10	Abraxane	7.3	1.7	SPARC
2014	Soares [16]	43	21	22	Capecitabine; docetaxel	5.3	3.7	TP
2014	Ban [17]	33	30	3	Belotaxel; belloxa	10.9	3.6	Double-strand breaks in DNA
2014	Borad [18]	69	40	29	TH-302 with gemcitabine;	6.9	3.6	Severe hypoxia Intracellular reductases
					Gemcitabine			
		71	44	27	TH-303 with gemcitabine;	8.7	5.6	
					gemcitabine			
		74	42	32	TH-304 with gemcitabine;	9.2	6	
					gemcitabine			
2015	Phan [19]	32	22	10	Pazopanib hydrochloride	25	14.4	VEGF
		20	12	8	Pazopanib hydrochloride	18.5	12.2	
2019	Wang-Gillam [20]	151	87	64	MM-398	4.9	2.7	Topoisomerase I inhibitor
		149	81	68	5 Fluorouracil; leucovorin	4.2	1.6	
		117	69	48	MM-398; 5 fluorouracil;	6.2	3.1	
					leucovorin			

Table 2.
Clinical trials with a successful outcome on pancreatic cancer.

Year	Author	N	M	F	Drug	OS	PFS	Biomarkers
2008	Spano [21]	69	35	34	Gemcitabine	5.6	3.7	VEGF
		34	16	18	Gemcitabine; AG-013736	6.9	4.2	
2015	Hobday [22]	58	29	29	Bevacizumab; temsirolimus	34	13.2	VEGF
2016	Stein [23]	37	21	16	MPC modified FOLFIRINOX	10.2	6.1	DPD
		31	20	11	LAPC modified FOLFIRINOX	26.6	17.8	

Table 3.
Clinical trial with neutral outcomes on pancreatic cancer.

5.2 Estimation of clinical trials with the neutral outcome on advanced pancreatic cancer

Here are three studies clarified neural results in OS and PFS result, ranged from 2008 to 2016, totally recruited 229 APC patients (121 male and 108 female). For details please see **Table 3**. Averagely the OS is 16.6 months and PFS is 9 months in neutral studies. For example, Jean in 2008 reported gemcitabine plus AG-013736 achieved better OS and PFS (6.9 and 4.2) than gemcitabine single used.

5.3 Estimation of clinical trials with the failed outcome on advanced pancreatic cancer

Regarding the failed outcomes, there are 14 studies ranged from 2011 to 2018, recruited 2448 APC patients (1385 male and 1063 female) for clinical trials. For details please see **Table 1**. Averagely the OS is 8.25 months and PFS is 4.39 months. It is evident that Faivre et al. in 2016 tested sunitinib malate to treat APC and achieved 38.6 months of OS and 12.6 months of PFS. However, Brian reported that hydroxychloroquine cures APC, unfortunately, there were 1.8 months both in OS and PFS negativity. Even though many combination tests, the benefit for APC patient is low.

6. Different perspectives on biomarkers

6.1 Vascular endothelial growth factor, VEGF

VEGF is a highly specific vascular endothelial cell mitogen and an angiogenic factor associated with platelet-derived growth factor (PDGF) structure. It is also known as vascular permeability factor (VPF), due to its permeabilization of blood vessels [24]. It is a subfamily of growth factors and belongs to a family of platelet-derived growth factors of cystine knot growth factor. VEGF is divided into the following groups: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor. VEGF binds to three transmembrane receptors (VEGFR1, VEGFR2 and VEGFR3). This receptor initiates downstream signaling through intracellular tyrosine kinase activity [25]. In fact, VEGF family members are playing an important role in the physiological angiogenesis of adults. Like wound healing, ovulation and pregnancy [26].

Activation of the VEGF/VEGF receptor (VEGFR) axis may trigger multiple signaling networks. Consequently, this may lead to endothelial cell survival, mitosis, migration and differentiation, vascular permeability and mobilization of endothelial progenitor cells (EPCs) from the bone marrow into the peripheral circulation [27]. On ligand binding, VEGFR-2 dimerization results in kinase activation and autophosphorylation of tyrosine residues. Activation of PKC may stimulate the Raf/MEK/ERK pathway, which accelerates the cell proliferation. Ca^{2+} mobilization and PKC activation are playing the key role in signaling events for VEGF-A-induced vascular permeability through activation of endothelial nitric oxide synthase activity [28].

The sword has double sides. VEGF participates in the pathogenesis of cancer, proliferative retinopathy and rheumatoid arthritis [29]. Its antibodies have shown therapeutic potential to inhibit tumor growth in vivo by inhibiting tumor-induced angiogenesis [30]. VEGF overexpression is associated with a variety of tumor progression and poor prognosis, including colorectal cancer [31], pancreatic cancer [32],

gastric cancer [33], breast cancer [34], lung cancer [35], prostate cancer [36] and melanoma [37]. This unique protein aids tumors grow and can be used for cancer treatment if used properly.

Early in 1971, Folkman first proposed the idea of angiogenesis. He believed that tumor growth and proliferation are closely related to angiogenesis and could be used as a targeted tumor therapy procedure [38]. Subsequently, countless scientists gathered in the field of VEGF to study the molecular mechanism of VEGF in tumor angiogenesis. They have also used it as a drug target to block the formation of blood vessels, thereby inhibiting tumor growth [39]. Studies have shown that the mechanism of anti-VEGF inhibitors may involve a variety of signaling pathways, such as FGF, D114, PGF/VEGFR1 and VEGF-C/VEGFR2. At least some of these pathways can increase the efficacy of VEGF inhibitors [40]. The anti-tumor drugs were developed with VEGF as the main target area. Like anti-VEGF humanized monoclonal antibody, VEGF-targeted antibody, protein kinase inhibitor and tumor vaccine [41, 42]. However, in the clinical application of anti-antigenic drugs, reliable biomarkers have not been found to screen the target population before patient improvement.

Our data disclosed that there were two studies reporting successful outcomes [14, 19], two studies reported neutral outcomes [21, 22] and four studies reported negative outcomes [43–46]. The results from the above eight trials remain controversial.

Regarding successful outcomes, Raymond et al. found that neuroendocrine tumors may be particularly sensitive to the combined inhibition of VEGFR and PDGFR. As for the neutral outcomes [14], Spano et al. used the gemcitabine + axitinib to treat the APC [21]. However, results differ from the results of Phase III trials in which erlotinib + gemcitabine confers the greatest survival advantage for patients with ECOG status 2 and metastatic disease, it possibly due to the mechanism of action between different EGFR and VEGFR inhibitors. Moreover, Hobday et al. found that the median PFS in the gefitinib trial was only 3.7 months which was even lower than the placebo group in the phase III trial [22].

With respect to the failed outcomes, Kindler et al. found that the effect of axitinib and gemcitabine on APC was limited to improve the survival period in patients with locally APC [43]. In fact, the results may be related to the gene locus of the VEGF receptor 1 tyrosine kinase domain. Furthermore, Ropugier et al. found that PFS was not significantly improved between the treatment arms. It indicates that blocking the VEGF/VEGFR axis does not lead to the survival of a patient with APC [45]. Nooan et al. study shows that pelareorep combination chemotherapy is not a sufficient solution to overcome the severe immunosuppression prevalent in PCA patients [46].

6.2 Epidermal growth factor receptor, EGFR

EGFR is one of the transmembrane receptors of epidermal growth factor family members of extracellular protein ligands. Its main function is to regulate various cellular functions including proliferation, movement and differentiation. Its mechanism can be described as the binding of the ligand to EGFR leading to dimerization followed by autophosphorylation of EGFR and activation of downstream signaling pathways. Activation of EGFR triggers multiple signal cascades within the cell, ultimately leading to gene transcription and biological responses [47]. Recently, studies have shown that dimerization occurs even in the absence of ligands, particularly when EGFR is overexpressed, possibly limited to a subset of dimers. Moreover, overexpressed EGFR can dimerize and become tyrosine phosphorylated without ligand [48].

EGFR signal transduction pathway can directly participate in tumor pathogenesis and progression [49]. EGFR overexpression plays a major role in carcinogenesis in cancer development [50]. Overexpression of EGFR accounts for 90% in pancreatic cancer cells [51]. Specifically, the aberrant activity of EGFR may impact the development and growth of tumor cells [52].

Mutation of EGFR may induce the resistance of tyrosine kinase inhibitors (TKIs) [53]. Notably, in tumor angiogenesis, vascular endothelial growth factor receptor-2 (VEGFR-2) plays a key role, and inhibition of VEGFR-2 signaling pathway has become an attractive cancer treatment method [54]. The binding of VEGF to VEGFR-2 stimulates the signaling pathway (PI3K/Akt, p38MAPK) that mediates several cellular functions. Besides, glycoproteins, EGFR and VEGFR-2 are in close correlation. Inhibition of EGFR can also reduce VEGF expression, while VEGFR-2 targeting can enhance the anticancer activity of EGFR inhibitors. Therefore, the dual inhibition treatment of EGFR and VEGFR-2 has a good effect and represents a promising cancer treatment. Recently, several EGFR/VEGFR-2 dual inhibitors have been discovered, such as vandetanib showing effective inhibitory activity on EGFR and VEGFR-2 [55].

A receptor tyrosine kinase is associated with cell proliferation and survival. Epidermal Growth Factor Receptor, EGFR, is overactive in many epithelial-derived tumors. It has been reported that EGFR is not related to its kinase activity, but rather maintains basal intracellular glucose levels to prevent autophagic death of cells. Despite the presence of chemotherapeutic drugs and tyrosine kinase inhibitors, this function of EGFR allows tumor cells to have higher viability [56]. EGFR inhibitors for cancer therapy are rapidly evolving in the broad context of cancer therapy, and in those patients achieving significant tumor response to EGFR inhibitors. Most patients will eventually exhibit disease progression, suggesting acquired resistance. This reminds us that increasing our ability to recognize tumors that depend on EGFR signaling growth is critical for the best choice of treating patients [57].

Unfortunately, two clinical trials showed that the results failed via EGFR. Studies by Ko et al. showed that subjects treated by cetuximab, bevacizumab and gemcitabine had 5.41 months in OS and 4.17 months in PFS. Propper et al. in 2014 tested Erlotinib to treat APC, and only achieved 4.0 months of OS and 1.5 months of PFS, **Table 4** [44, 58]. Ko reported the incidence of severe (grades 3–5) toxicity, comparable to the use of gemcitabine as a single agent in this patient population which may reflect a relatively short duration of treatment due to lack of efficacy. Moreover, it is difficult to explain the quality of life analysis based on the number of patients completing the series of questionnaires and the overall time the patients stayed in the study [44]. Propper concluded that there is limited evidence to support the use of predictive biomarkers for patients with pancreatic cancer who could benefit from targeted therapies [58].

6.3 Kirsten rat sarcoma viral oncogene homolog, KRAS

KRAS protein plays a key role in human cancer but has not yet succumbed to therapeutic attacks [59]. The search is now focused on targeting alternative pathways that are activated in mtKRAS cells, to circumvent or prevent drug resistance [60].

There are currently no therapeutic interventions for KRAS. Pharmacological agents that are speculated to inhibit KRAS include farnesyltransferase inhibitors that block the binding of KRAS to the cell membrane. These drugs have failed in clinical studies. Antisense oligonucleotides and engineered microRNAs (miRNAs) have been used as an alternative to targeted mutations in KRAS without disrupting

Year	Author	N	M	F	Drug	OS	PFS	Biomarkers
2011	Kindler [43]	314	191	123	Gemcitabine; AG-013736	8.5	4.4	VEGF
		316	188	128	Gemcitabine; placebo	8.3	4.4	
2012	Ko [44]	29	18	11	Cetuximab; bevacizumab gemcitabine	5.41	4.17	EGFR VEGF
		29	14	15	Cetuximab; bevacizumab	3.55	1.91	
2013	Rougier [45]	275	157	118	Placebo; gemcitabine	7.8	3.7	VEGF
		271	160	111	Aflibercept; gemcitabine	6.5	3.7	
2013	Wu [61]	30	16	14	Etanercept; gemcitabine	5.43	0.3	TNF-α
		8	3	5	Gemcitabine	8.1	1.8	
2014	Propper [58]	104	59	45	Erlotinib	4.0	1.5	EGFR
		103	59	44	Placebo	3.1	1.5	
2014	Infante [62]	80	39	41	Gemcitabine; GSK1120212	8.4	16.1	cfDNA
		80	46	34	Placebo; gemcitabine	6.7	15.1	
2014	Wolpin [63]	10	5	5	Hydroxychloroquine 400 mg	1.8	1.8	LC3-II
		10	6	4	Hydroxychloroquine 600 mg	3.0	1.6	
2015	Catenacci [64]	53	27	26	Gemcitabine hydrochloride; Placebo	6.1	2.5	SHH
		53	31	22	Gemcitabine hydrochloride; vismodegib	6.9	4.0	
2016	Noonan [46]	36	22	14	wild-type reovirus; carboplatin; paclitaxel	7.31	4.94	VEGF; IL-6; IL-8
		37	19	18	Carboplatin; paclitaxel	8.77	5.2	
2017	Chung [65]	62	22	40	Fluorouracil; oxaliplatin	6.7	2.0	KRAS protein
		58	35	23	Akt inhibitor MK2206; selumetinib	3.9	1.9	
2017	Faivre [66]	86	42	44	Sunitinib malate	38.6	12.6	VEGF; PDGF
		85	40	45	Placebo	29.1	5.8	
2017	Ko [67]	66	38	28	OGX-427	6.9	3.8	Hsp27
		66	37	29	Placebo	5.3	2.7	
2017	Laquente [68]	65	42	23	LY2603618; gemcitabine	7.8	3.5	CHK1
		34	20	14	Gemcitabine	8.3	5.6	
2018	Van Cutsem [69]	44	22	22	Gemcitabine; placebo	7.6	2.8	ERK 1/2
		44	27	17	Gemcitabine; pimasertib	7.3	3.7	

Table 4.
Clinical trial failed outcome on pancreatic cancer.

the expression of non-mutant KRAS and have achieved some success in preclinical trials [70].

In recent years, many studies have suggested that the oncogene KRAS plays a major role in controlling cancer metabolism by coordinating multiple metabolic changes [71]. Furthermore, combined inhibition of therapeutic effects and feedback pathways is promising in KRAS mutant cancers. Moreover, it is unclear what specific pathways should be used to optimize treatment response [72].

Unfortunately, Chung et al. tested pharmaceutical target via KRAS protein, whereas the result was unsuccessful. The results showed that the OS and PFS of patients treated by Akt Inhibitor MK2206 and selumetinib were 3.9 and 1.9 months, respectively, see **Table 4** [65]. The results indicated that the strategy of utilizing two or more kinase inhibitors is subject to the challenge of toxicity overlap. These toxicities will significantly block the delivery of effective inhibitory amounts of both drugs in vivo. However, a major disadvantage factor is the delay in toxicity-related treatment and the frequency of dose reduction in the experimental group through damaging sustained signal suppression [65].

7. Conclusion

APC reserves unpredictable mechanisms to maintain a highly resistant phenotype. The genetic and epigenetic alterations of the APC lead to the resistance of the chemotherapy.

Nowadays, many biomarkers have been on board to improve the clinical treatment outcome of advanced pancreatic cancer. Although these successful biomarkers have provided notable therapeutic effects on advanced pancreatic cancer, the outcomes remain unsatisfactory to the patients and health providers. With the development of the biology of advanced pancreatic cancer, we now expect better biomarkers and conduct therapy by unveiling the tumor microenvironment and the mechanism of the mutations (**Figure 1**).

We can assume that with the development of truly effective treatments and clinically useful markers for early detection of the disease, better combination of markers to advanced pancreatic cancer. In the meanwhile, researchers are trying to detect magnificently predictive biomarkers to decide the treatment strategy and permit practitioners to adequately evaluate and propose individualized treatment protocols which would give a greater survival rate.

Clearly, there is a need to better understand the underlying signaling networks that drive pancreatic cancer progression and potential escape mechanisms. In addition, it is necessary to improve the role of preclinical models of pancreatic cancer

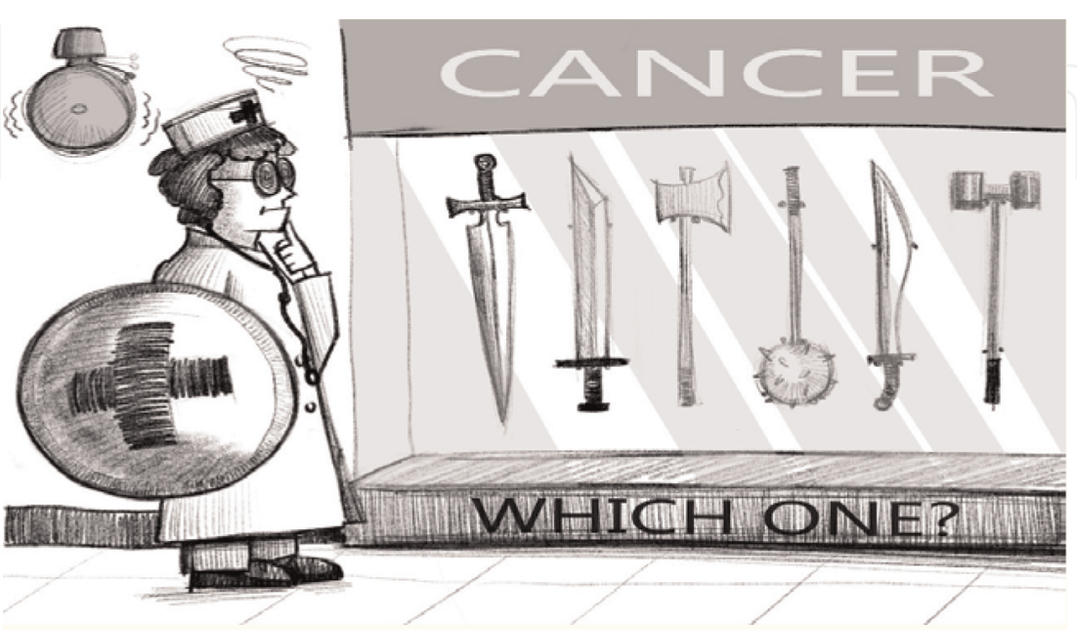

Figure 1.
Difficulties in decision making both for R&D and doctors.

and the optimal transformation of preclinical success into experimental design. The genetic and proteomic technologies show great potential to detect the novel biomarkers in cancer research. We place great expectations on these technologies to personalize treatment for advanced pancreatic cancer patients.

Acknowledgements

This work was supported by the Chengdu Medical College Natural Science Foundation (CYZ18-08, CYZ18-20, CYZ18-33), the Sichuan Provincial Education Department (17ZA0134), the Sichuan Medical Association (S18023), and Sichuan undergraduate innovation and startup program funding support (S201913705080, S201913705130, S201913705059).

Authors' contribution

XF developed the general idea and drafted the manuscript with CJ; YLQ and DYX searched the reference, HK sort the clinical trials data, PTT and ZS participated in drafting process and double check the data; LFF drew the figure; GM proofread the manuscript and ensure the general quality.

Competing interests

The authors declare that they have no competing interests.

Ethics statement

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data will be made available upon reasonable request.

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