

## Personalized Targeted Prevention and Therapy Relied on Detection of Global and Local Single Nucleotide Polymorphisms

David Deng, Biaoru Li\*

Department of Pediatrics, Children Hospital, Augusta, USA

\*Corresponding author: Li, B. Department of Pediatrics, Children Hospital, Augusta, USA. E-mail: [BLI@gru.edu](mailto:BLI@gru.edu)

### Abstract

Personalized medicine, one of special prevention and therapeutic strategies, is going to develop into clinical fields. Personalized medicine is directly tailored for physicians to prevent and care individual patient. It is often called as “the right treatment for the right person at the right time.” Most successful examples of personalized prevention and treatments require a rational clinical genomic analysis. Following Research and Development (R&D) of clinical genomic techniques, here we introduce personalized targeted-therapy based on either detection of single nucleotide polymorphisms (SNP) or detection of universal single nucleotide variance (SNVs) at genomic level. According to clinical protocol of personalized targeted prevention and therapy, whole performance includes clinical sampling, SNP detection technology and diagnosis (universal and designed), SNP signature discovered by system modeling and sensitive targeted molecules/drugs uncovered by drug-banks with its confirmation. Now, after (A) simplified and designed SNP detection test is applied for clinical patients and (B) next generation sequencing for universal SNP detection is brought into the new field, both universal and designed SNP detection systems related with sensitive targeted molecules will make great contribution for further personalized prevention and treatment of tumor diseases, genetic diseases and uncured disease in nerve, endocrine and cardiovascular systems.

**Keywords:** Genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP), transcriptome, Encyclopedia of DNA Elements (ENCODE), flow-cytometric cell sorting (FACS), magnetic cell separation (MACS), Laser-captured micro-dissection (LCM), clinical genomics analysis and diagnosis, system biology and system modeling, networks, personalized medicine, personalized target therapy.

### Introduction

Some diseases including tumor, genetic diseases and uncured diseases in nerve, endocrine, cardiovascular systems require physicians to address a question about effective prevention and treatment<sup>[1]</sup>. This is why physicians should apply for new techniques available as soon as possible. Now some important discoveries in enhancing the therapeutic effect have emerged in clinical fields such as “personalized medicine”<sup>[2]</sup> and “targeted therapy”<sup>[3]</sup>. Personalized medicine is a new medical model relied on individual characteristics of each patient rather than “one size fits all” treatment. Detecting patient’s genomics profile is an important foundation of personalized medicine. This genomic profile can be applied for physicians to define what genomics

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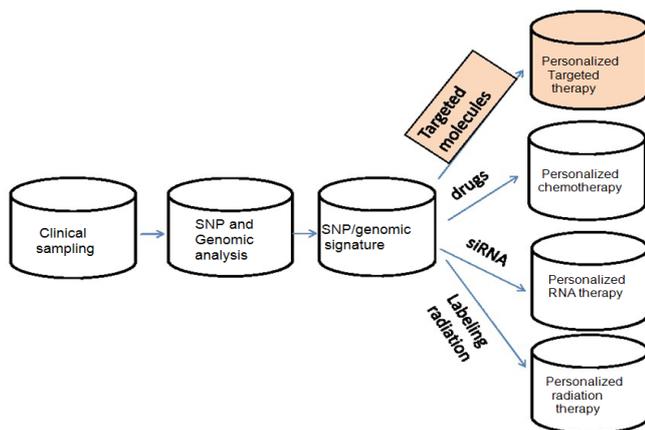
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change makes the patient susceptible to his disease or anticipates which medical prevention and treatments will be safe and effective for the patient<sup>[4]</sup>. Recent development of clinical research has enabled physicians to understand causes and mechanism of some diseases related to genomic profiles analyzed by genome-wide association studies (GWAS) for single nucleotide polymorphisms (SNP) of different prevention and treatment<sup>[5]</sup>. Targeted therapy (also called as molecularly targeted therapy) is second advanced module by interfering with specific targeted molecules needed for some diseases such as carcinogenesis and tumor growth rather than by simply interfering with all dividing cells (as traditional chemotherapy) or some stimulating with specific inducing molecules needed for some other diseases such as diabetes or genetic disease rather than by simply inducing within all cells. Because most clinical application of the agents have some toxicity to normal cells, such as agents for tumor disease called as chemotherapy, if both modalities (personalized therapy and targeted therapy) can be



combined, both personalized therapy (based on SNP of individual response for each agent) and targeted therapy (based on small molecules for specific targeted molecules expected to “higher selective targeting” and “less toxic” than other personalized therapy) will be more effective than other forms of treatments. The combination, here, is called as Personalized Targeted Prevention and Therapy (PTPT or Personalized Targeted Therapy, PTT). Moreover, scientists are going to integrate SNPs profiles with other genomic profiles among of them (GWAS-transcriptome, GWAS-microRNA, GWAS-transcriptome-epigenetics, All genomics profiles GWAS-epigenetics-microRNA-transcriptome-proteomics or Encyclopedia of DNA Elements, ENCODE) to study therapeutic targeting<sup>[6]</sup>. At present, if the PTPT combinations are successful, the module with two features, “personalized” and “targeted” can be developed into prevention and treatment of most diseases including nerve-cardiovascular, tumor, genetic and uncured diseases. In the near future, PTPT will play an important role in ENCODE data. In order to introduce rational Personalized Targeted Prevention and Therapy (PTPT), according to workflow of Personalized Targeted Prevention and Therapy (Figure 1), In the review manual, we will first introduce (A) clinical sampling for SNPs and genomic techniques and then present (B) clinical SNP detection related analysis and diagnosis; discuss in detail (C) SNP signature related system model including system model concept, current system model for personalized prevention and therapy and clinical application; finally, we will briefly list current targeted therapy and present (D) personalized targeted prevention and therapy related with different validation methods. In conclusion section, we will address future development of SNP related system model for Personalized Targeted Prevention and Therapy.



**Figure 1.** the diagram of procedure of personalized therapy includes: A. clinical sampling; B. clinical SNP and genomics diagnosis; B. SNP signature/GES mining; C. personalized therapy regarding personalized targeted prevention and therapy by small molecules, personalized chemotherapy, personalized RNA therapy and personalized radiation therapy. Pink color is main part in the manual.

### Clinical Sampling for SNP And Genomic Analysis

Mixed-cells from clinical samples are greatly challenges for genetic diagnosis and genomics data analysis. In order to achieve objective results of genetic diagnosis and genomics data analysis for personalized target prevention and treatment, we will first address a question about SNP and genomic analysis from clinical samples. In clinical genomic analysis from reported evidences, several sampling techniques have been applied

for clinical SNP and genomic analysis<sup>[7,8]</sup>: clinical sampling *in vitro* (single-cell sampling for SNP and genomic analysis), clinical sampling *ex vivo* (purifying/expanding primary cells *ex vivo* from clinical samples for SNP and genomic analysis) and direct clinical SNP and genomic analysis *in silico* (SNP and genomic analysis using different bioinformatics model to observe SNP and genomic data from tissue level).

### Clinical sampling in-vitro

Clinical sampling *in vitro* includes clinical cells isolation consisting of flow-cytometric cell sorting (FACS)<sup>[9]</sup>, magnetic cell separation (MACS)<sup>[10]</sup> and laser-captured micro-dissection (LCM)<sup>[11]</sup> with downstream SNP and genomic analysis. FACS can isolate clinical cells by a specific biomarker on the cell surface such as CD34 cells for stem cell, CD3 for T-cell, CD71 for early erythroid cells, CD133/CD34 for cancer stem cells (CSCs)<sup>[12]</sup> and EpCAM for circulating tumor cells (CTCs)<sup>[13]</sup>. At present, multi-coloured FACS can specifically harvest identified cells in a vial by combined biomarkers so that FACS can enhance its ability to mine SNP and genomic profile. MACS technique, the second cell harvesting technique *in vitro* for clinical genomics analysis, is often used to sort primary cells by cell-surface biomarkers as FACS technique. At present, MACS can use multi-labelling Abs to negative or positive select identified cells with surface biomarkers; therefore, it also can increase its ability to uncover SNP and gene profile in a given cells at tissue level. Among LCM techniques, LCM can achieve clinical cells based on morphology change on glass slides or relied on specific mRNA/protein biomarkers. LCMs can specifically harvest clinical cells *in vivo* environment<sup>[14]</sup>. LCMs also can combine Ab-based stains and DNA/RNA FISH stains to increase the cell specificity from their biomarkers. In these several years, along with R&D of LCM techniques and biomarker identification for primary cells, LCM has been quickly developed (A) from fixed cells into living cells which can be further cultured for primary cells due to downstream genomic analysis<sup>[15]</sup>; (B) LCM can be used for automation system for high-throughput screening<sup>[16]</sup>.

### Clinical sampling ex-vivo

Clinical sampling *ex vivo* includes clinical primary cell culture with downstream SNP and genomic analysis. In 1977, Drs. Hamburger and Salmon first set up clinical tumor-cell culture to assay drug sensitivity from tumor patients<sup>[17]</sup>. In 1994, 50 cases of primary tumor-cell culture were reported for drug sensitivity assay in our reports<sup>[18]</sup>. Now the techniques to increase primary cell number to perform clinical genomic and SNP analysis have been largely presented. Along with R&D of primary cell culture, stem cell, CSC and other primary cells cultured with downstream genomic analysis are going to play an important role in personalized therapy. Furthermore, *ex vivo* sensitivity-assay of cultured cell system can verify suggested targeted molecules for personalized therapy.

### Clinical SNP and genomic analysis in silico

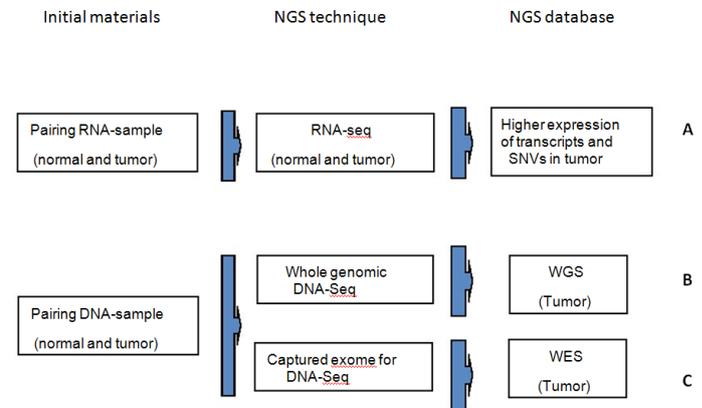
In the surgical and gynecological field, most clinical specimens are directly frozen at tissue level after surgical removal. If the specimens are processed by SNP microarray and NGS at the tissue level, genomic analysis *in silico* is a very important performance for clinical SNP and genomic analysis because

the SNPs and genomic data are mixed with different SNPs and genome profiles from the mixed cells. According to published data<sup>[19]</sup>, two combined groups of bioinformatics techniques can increase purity of clinical genomic analysis from the mixed cells: (A) tissue-level by hierarchical cluster, principle component analysis (PCA), and self-organizing map (SOM) and (B) molecular-level by supervised learning based on cell-biomarkers and time- courses relied on cell-biomarker course-change.

### Clinical SNP detection and genomic analysis

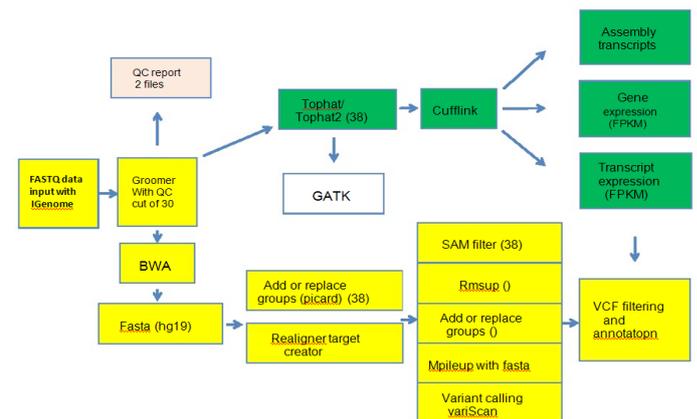
SNPs are the most common types of genetic variation in all genetic variation. An SNP is a single base pair mutation at a specific locus, usually consisting of two alleles (where the rare allele frequency is  $>1\%$ )<sup>[20]</sup>. SNPs in the Hap-map will provide the minimal set of SNPs needed to genotype of human disease. SNPs involved in development of many human diseases become particular benefits for pharmacogenetics with down-stream individual therapy. SNP detection is single nucleotide polymorphisms measurement between disease sample and control sample from patient. In order to clearly explain performance of PTPT, here, we conclude some detection assays including SNPs profiles defined by global approaches (universal SNP detection at genomic level) and local approaches (designed SNP genetic testing) as below. In global SNP analysis several new techniques have been employed into universal SNPs detection or global genomic level including (A) SNP microarray hybridizing complementary DNA probes to the SNP site and (B) next-generation sequencing technologies to read whole genomes by grouping samples<sup>[21,22]</sup>. SNP microarray is high-density oligonucleotide SNP arrays with hundreds of thousands of probes arrayed on a small chip to detect universal SNPs from a pair of clinical DNA sample. At present, Affymetrix and Illumina Inc. still are available for these kinds of products such as Human SNP 5.0 Gene Chip can genotype over 500,000 human SNPs. Because SNP alleles only differ in one nucleotide and because it is difficult to achieve optimal hybridization conditions for all probes on the array, mismatched probes are potential challenge for the target DNA. In order to address mismatch problem, current designed microarray probes from commercial companies have the SNP site with several different control containing mismatches in the SNP allele. By comparing the differential amount of hybridization of the target DNA to each of these redundant probes, it is possible to determine specific homozygous and heterozygous alleles. Next generation sequencing (NGS) is second universal SNP detection system currently and quickly developed due to more throughput than SNP microarray so that it can use single nucleotide variants (SNV) calling from NGS data to identify all SNVs in pairing specimen. As our several publications and published book, three NGS technologies, whole genomic DNA-Seq (WGS), whole exome-Seq (WES) and RNA-Seq all can be used to detect SNPs as Figure 2<sup>[23-25]</sup>. Computational techniques as Figure 3 have been successfully adopted to identify rare SNPs within a comparison samples and to detect SNVs within an individual using multiple tissue samples<sup>[26]</sup>. These three NGS to detect universal SNVs have different advantages and disadvantages. For example, RNA-Seq can detect both transcriptome and SNVs (Figure 2A) which can be benefits for downstream system model for SNP signature discovery although the initial material is RNA which is easy to be disrupted; for DNA-Seq (both WGS and WES), initial materials are DNA which is very stable and

easy to amplify. WGS (Figure 2B) is a most throughput technique to detect all SNVs including coding and non-coding DNA sequences while WES (Figure 2C) is cost-effective than that of WGS with about 2% WGS key information thus lower throughput than WGS.



**Figure 2.** Next-generation sequencing includes:

- A. pairing RNA-seq from tumor-normal cell;
- B. pairing whole genomic sequencing (WGS) from tumor-normal cell;
- C. pairing whole exome sequencing (WES) from tumor-normal cell.



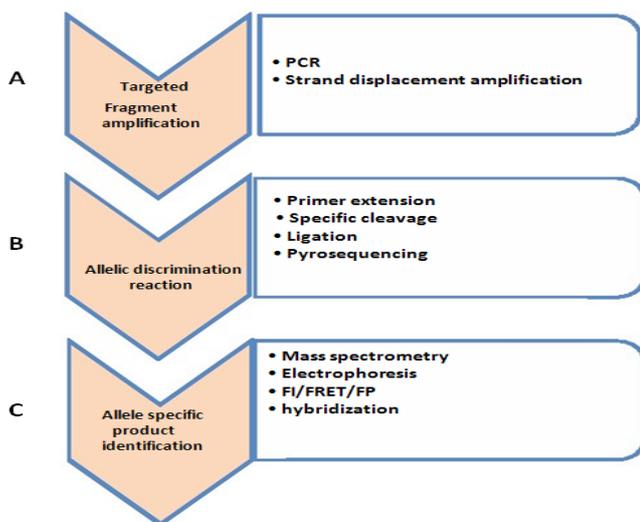
**Figure 3.** the diagram of procedure of NGS includes: A. transcriptome mining process as green color;

B. SNVs mining process as yellow color.

### Designed SNP detection

A set of SNPs for leukemia was patented in Dr. Preisler laboratory for whom we worked in 2002. After that, many new technologies for SNP genotyping setting have been developing for fifteen years. Now, if we know that a set of SNPs or chromosomal region are related to a disease, company kits with their products can be easily used for SNP detection in different clinical laboratories. Accordingly, designed SNPs genotyping protocols include three steps as Figure 4<sup>[26]</sup>: target amplification, allelic discrimination and product detection/identification. Target DNA Amplifications use almost all the polymerase chain reaction (PCR) techniques with their  $10^9$  copies amplification excepting invader technology. Allelic discriminations are a key of SNP genotyping. The discriminating power generating from DNA polymerases and DNA ligases can differ matched and mismatched DNA duplexes with high specificity and accuracy. Allele discrimination can be used by hybridization, ligation and the 5' nuclease activity of DNA polymerases with the combination of their PCR that created the Molecular Beacons, the Taq Man

assay and the FRET-DOL assay. These single-steps in one well/one tube assays can simplify protocol applied for automation. The specificity and accuracy of the methods are mainly depended on enzymes: best specificity ranking from DNA ligases, endonuclease to allele-specific hybridization although DNA polymerases vary in specificity based on the different enzyme activities. The final step is the detection and identification of allele-specific products. After an additional amplified DNA purification, product detection and identification can be performed by mass spectrometry, fluorescence resonance energy transfer (FRET), fluorescence polarization (FP), luminescence, absorbance and melting temperature. Most detection systems have built-in mechanisms to perform repetitive work for many samples, such as 96- and 384-well plates or hybridization on a solid membrane<sup>[28-31]</sup>. Because so many products are available on the markets, clinical scientists and physicians are difficult to select appropriate one for their applications. In this manual, we also concluded some SNPs techniques and kits with their strengths/weaknesses, ranking/throughputs and cost range of these technologies for clinical application as a Table-1. Of course, our important points are to focus on therapeutic application of SNP detection described as the next section.



**Figure 4.** the diagram of procedure of designed SNP detection includes:  
A. Targeted Fragment amplification by PCR;  
B. Allelic discrimination reaction by ligase, cleavage and extension;  
C. Allele specific product identification by mass spectrometry, electrophoresis, fluorescence and hybridization.

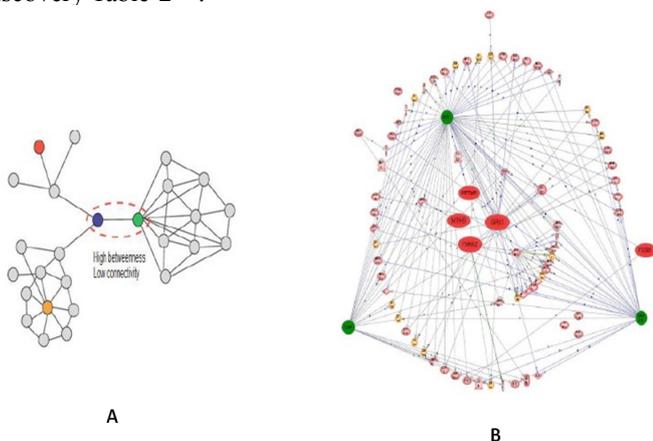
**Table 1:** Comparison of routine SNPs detection kits for PTPT

Types	Methods	Scales (SNP No)	Samples	Strength	Weakness	Cost
Local	Taq Man assay 4	1-10	96-384	Simple and easy to automate	lower throughput	0.6 per SNP/genotype
	Sequenom	10-300	50-1000	Medium throughput	Higher cost for equipment	0.2 per SNP/genotype
	Illumina GoldenGate	384 — 3072	96 -1000	Medium throughput	Higher cost for equipment	0.2 per SNP/genotype
	Captured-Seq	targeted all SNPs-disease	depending	Higher throughput for a given	Higher cost equipment	depending on sample size
Global	SNP Microarray	Universal	12	higher throughput	Costly equipment and bioinformatics analysis	expensive
	WGS	Universal	1	coding and non-coding	Costly equipment and bioinformatics analysis	expensive
	WES	Universal	1	exome	Costly equipment and bioinformatics analysis	expensive
	RNA-Seq	Universal	1	SNVs and transcriptome	Costly equipment and bioinformatics analysis	expensive

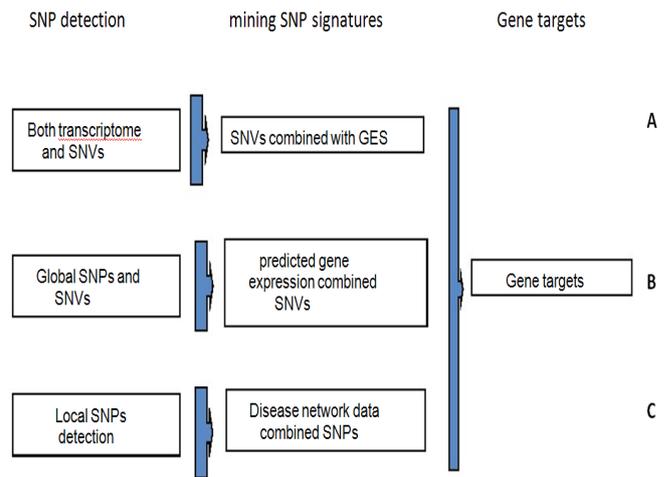
### SNP Related system modeling

After SNPs are detected by local and global methods, especially SNVs are mined from NGS, if the SNPs are used to predict the patient susceptible to his/her disease, patient SNPs information comparing genome-wide association studies (GWAS) Database with their population analysis provide a first step of powerful approach to identify disease loci<sup>[32]</sup>. GWAS pairing case-control samples with a statistical analysis determine whether the alleles at the marker can predict the phenotype. If GWAS reaches statistical significance after multiple testing, the variant is considered to be associated with the diseases. Now disease susceptibility has been successfully conducted by GWAS using risks prediction and population analysis. Following comparing patient's universal SNVs to GWAS to identify SNP related with a given disease, functional SNPs techniques are further studied for causal SNP discovery (use platforms such as POLYPHEN, POLYPHEN-2, SIFT and MUTATIONAL TASTER) with clinical design (such as male vs female for SNPs in XY chromosome, some clinical features and phenomenon related function SNPs)<sup>[33]</sup>. GWAS SNVs related to disease susceptibility and functional SNPs associated to disease-cause play an important role in prediction and precaution of diseases. However, most of therapeutic agents and targets are directed at phenotype alteration, such as function change of tumor cell related with mRNAs and proteins expression level, rather than on DNA SNP informative archives<sup>[34]</sup>. If SNPs profiles are combined by system biology based on gene expression and phenotype alteration of a given disease, therapeutic targets will be definitely discovered by us-

ing the SNPs or SNVs. Here, in order to determine SNPs related to therapeutic targeting, we will introduce system biology in detail, and then explain network regarding topology analysis and its application for personalized targeted therapy. Finally, we will briefly present global and local SNP detection related with the therapeutic targeting. Systems biology is a mathematical modeling of complex biological systems. These biological complex interactions generally involve in metabolic networks or cell signaling networks<sup>[35]</sup>. The systems biology is ability to better diagnose and better predict the outcome of a suggested treatment for personalized medicine. Systems modeling is the interdisciplinary method to construct systems and to guide a suggested treatment for personalized medicine<sup>[36]</sup>. Networks with their knowledge are foundation of system modeling with intracellular components as nodes and their interactions as links within the system modeling<sup>[37]</sup>. Different types of intracellular molecular and biological networks can be represented by different types of give diseases. The topology of networks can be “simulating-engineer” directly from gene quantity change. Currently, system modeling is performed by Cytoscape platform with plugin functional software to detect targeting genes<sup>[38]</sup>. Clinically, results of measurement will select targeting genes with best therapeutic effect for tumor cells and minimal toxicities for normal cells in the biological networks or high values of between centrality (BC) and low values of connectivity degree (CD) as effective gene targets as Figure 5A<sup>[39]</sup>. The resulting graphs represent topology type-I of targeting genes to perform personalized therapy analysis as Figure 5B<sup>[40]</sup>. Accordingly, SNP signatures linked to targeting genes can be discovered by three methods based on a given data obtained by their detection tests as Fig. 6: (1) Both SNPs and transcriptome profiles: For example, RNA-Seq can detect both transcriptome and SNVs. After gene expression signatures (GES) are mined by system modeling from transcriptome, SNP signatures, which is key to target gene related SNP, can be mined from the combination of GES and SNVs profiles<sup>[41]</sup>; (2) Only SNVs data detected by WGS and WES: SNPs signature is defined by a combination of SNVs profiles and functional genes related SNVs profiles<sup>[42]</sup>; (3) Only SNPs data detected by designed SNP assay: a combination of disease-network and designed SNP discovery Table-2<sup>[43]</sup>.



**Figure 5.** the diagram of system modeling by quantitative network: A. GES with higher “Betweenness” and lower “connectivity”; B. example of GES with higher “Betweenness” and lower “connectivity”. Red color is type-I targeting with higher “Betweenness” and lower “connectivity” and Green color is type-II with lower “Betweenness” and higher “connectivity”.



**Figure 6.** the diagram of SNP/SNVs signature mining includes: A. SNP detection by RNA-Seq, DNA-Seq and local SNP detection; B. SNP signature mining by three databases: SNP signature mining by both transcriptome and SNVs; SNP signature mining by combining DNA-Seq with predict gene expression; and SNP signature mining by combining local SNP detection with disease network; C. Gene targeting.

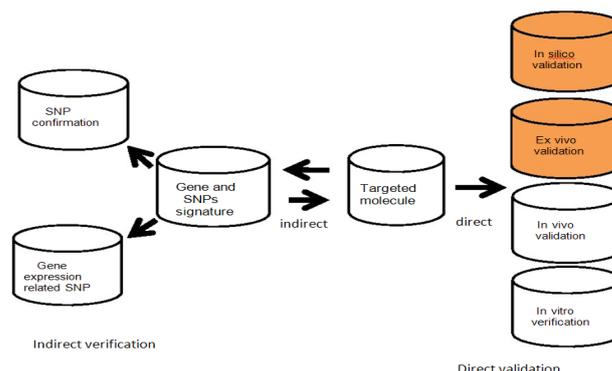
**Table 2:** Network Example in Some Tumor Diseases

Diseases	Pathway
Brain tumor	Notch; mTOR
Oral tumor	Wnt/β-Catenin Pathway
NSCLC	Wnt, EGFR Pathways
SCLC	Hedgehog signaling
Esophageal cancer	E2F-1
Stomach	NF-κB, Wnt/β-catenin, and proliferation/stem cell
Colon cancer	Wnt, CdhE, Prostaglandin, EGFR, TGF-BetaR, DCC
Liver cancer	Wnt/β-catenin signaling pathway, YAP and Hippo signaling pathway
Kidney cancer	VHL, VEGFR and mTOR, HGF/c-MET and Wnt/β-catenin
Bladder cancer	NOTCH pathway
Prostate cancer	Akt-regulated pathways
Ovarian cancer	Wnt/β-catenin pathway, AKT Pathway, PI3K/Akt/mTOR Pathway, MET/HGF Signaling Pathway
endometrial cancer	PI3K/Akt/mTOR Pathway, Wnt/β-catenin pathway
cervical cancer	Hedgehog
Breast cancer	HER2 Pathway, Ras/MAPK Pathway, (PI3) Kinase Pathway, p53 pathway
Thyroid Cancer	p16/p38MAPK/p53/Wipl pathway
Pituitary Gland Tumor	GH1 and ERK

### SNP Signature Related Targeted Therapy

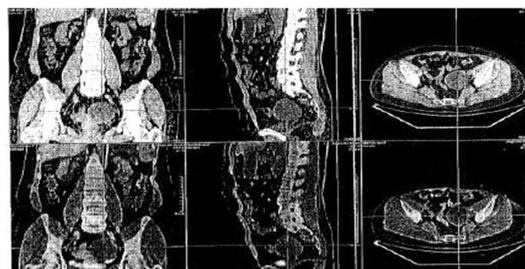
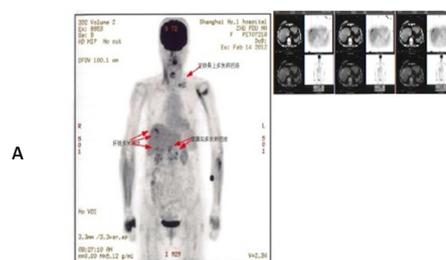
As we mentioned as above, DNA SNPs are informative archives although SNPs were largely reported to predict therapeutic targeting. Theoretically, genes uncovered by system modeling related with SNP signature can be directly used for all therapeutic targeting of personalized therapy including personalized chemotherapy, personalized RNA therapy and personalized radiation. Moreover, personalized targeted therapy is lower toxic than any other therapy forms as we discussed above, it can be used for both prevention and treatment. At present, Genecards, Drug-bank and commercial software such as GeneGo and Pathway Studio are routinely used to discover small

molecules, Abs and drugs<sup>[44,45]</sup>. Here, several targeted molecules often used in clinics are listed as Table-3. Although targeted molecules with their delivery system are quickly developed by pharmaceutical company and although targeting genes with their targeted molecules are discovered by SNP detection with its analysis of system modeling as well, we still require prudent application of personalized therapy for patients from bench to bedside. For example, whether suggested small molecules, Abs and drugs predict response to the patient's tumor-cells or not require to be confirmed. Now, increasing validation methods of antitumor agents were reported for personalized therapy<sup>[47]</sup>. According to published reports, here we list the validating methods including "indirect confirmation and direct validation" as Figure 7 and Table-4. Indirect confirmation: It is essential to identify SNP signature on drug-targeting such as Qrt- PCR and immunohistochemistry can be used to measure SNP related gene expression<sup>[48]</sup>. We have routinely added Qrt-PCR measurement as essential verification steps. Direct validation: Direct validation-assay has been successfully reported to predict outcomes of drugs efficacy including *in silico* module, *ex vivo* module, *in vivo* module and *in vitro* module. (A): *in silico* module: Network related to drug discovery have been largely reported. Dr. Zheng first set up python-based network to study tumor cell-line proliferation using scoring analysis<sup>[49]</sup>. In 2015, we further reported the model for a patient suffering from a triple-negative breast cancer (estrogen receptors, progesterone receptors, and HER2) with multiple metastases in liver, bone and other organs. Excitingly, after prediction and validation-mimic-assay by the python model, two groups' drugs were mined and confirmed for the patient, the patient achieved complete response after three months suggested drugs-treatment as Figure 8A,B<sup>[50]</sup>. (B): *ex vivo* module: As discussed above, clinical-cells cultures including primary tumor-cell have been set up to assay drug sensitivity by Drs. Hamburger and Salmon. Now several protocols of primary tumor cell culture with their techniques are going to apply for validation system of personalized therapy<sup>[51]</sup>. Thus *ex vivo* module demonstrates a good system to validate suggested compounds for personalized therapy. (C): *in vivo* module: xenografts of human cancer cells also is very good model to verify the different drugs. Some scientists developed xenografts of human cancer cells *in vivo* to validate compounds effect<sup>[52]</sup>. Although it is costly model, this model might become valuable model after reducing cost and routine service available in the near future. (D): *in vitro* module: Drug sensitivity of a particular agent in one kind of tumor could significantly impact decision treatment planning. Some scientists developed tumor cell-lines as model to verify classified response. For example, colorectal cancer for drug-resistance-based score systems can rank the patients' response to three first-line anticancer compounds, or 5-FU, oxaliplatin and irinotecan<sup>[53]</sup>. The gene patterns of these drug-resistant cell lines provide a strong basis to develop specific drug resistant gene signatures. As genetic SNPs in cancer cell lines contribute to mine the tested drugs, a special gene related to SNPs will support resistance mechanisms for the compounds. This model only can classifies therapeutic targets by genetic SNP determination in cancer cell lines.



**Figure 7.** the diagram of procedure of validation of personalized therapy includes:

A. indirect methods regarding GES confirmation by QrtPCR and protein levels; B. direct methods regarding *in silico* validation, *ex vivo* validation, *in vivo* validation and *in vitro* verification. Pink color is mainly use in clinical in current laboratories.



**Figure 8.** Results of positron emission tomography-computed tomography (PET/CT). Figure 8A shows multiple metastases before personalized therapy; Figure 8B indicates good response after 5 months' personalized therapy.

**Table 3:** Examples of Current Targeted Therapy

Types	Examples	Mechanisms	Current application
Tyrosine Kinase Inhibitors	Imatinib mesylate	BCL/ABL region	CML and GI tumors
	Gefitinib	EGFR	NSCLC
	Erotinib Salinomycin	EGFR cancer stem cell	NSCLC cancer stem cells
Serine/threonine Kinase inhibitors	Temsirolimus	mTOR	renal cancer
	Everolimus	mTOR	renal cancer, three negative breast cancer
	Vemurafenib Tametinib	B-Raf enzyme Mek	melanoma melanoma
Small molecule drug conjugates	Vintafolide	Folate receptor	ovarian cancer and NSCLC
Monoclonal Ab	Ritumab Trastuzumab	CD20 Her2/neu	NHL breast cancer
	Cetuximab	EGFR	colon cancer and NSCLC
	Bevacizumab	EGFR	breast, brain, colon and NSCLC

**Table 4:** Verification for compound sensitivity of therapeutic targeting

Methods	Technology	Advantages	Disadvantages
Indirect-confirmation	Qrt-PCR or ICC/IHC staining	Simple and cost-effective	Indirect data
<i>In silico</i> validation	Bioinformatics modules	Bioinformatics supports	Mimic data
<i>Ex vivo</i> validation	Primary tumor cell or CSC culture	Objective response for different drugs	Difficult techniques
<i>In vivo</i> validation	Xenografts of human cancer cells	Objective response for different drugs	Very costly and limiting time
<i>In vitro</i> confirmation	Tumor cell lines cultures	Simple and easy for different drugs	Non- individual patient

## Conclusion

Personalized medicine is a new medical model to treat individual patient relying on personal information including genomic data. If personalized therapy combines with targeted therapy, it will produce “stacking effect” called as personalized targeted therapy. Although personalized therapy including personalized targeted therapy based on a system modeling has been quickly developed, several questions still need to be addressed. Here we list several challenges which we should further answer and resolve: Clinical sampling needs more improvement: For example, (A) clinical sampling *in vitro* need to search and define more specific biomarkers to use for different clinical-cells; (B) clinical sampling *ex vivo* require developing easy-culture technique for primary cell growing-up. We have largely reported clinical primary-cell culture techniques including primary tumor cell, CSC and clinical stem cell for more than 25 years. Because primary cell culture technique is very complicated, such as different culture-conditions harvested from before-chemotherapy and after- chemotherapy, it needs more skilled expert for clinical laboratories. Fortunately, UT MD Anderson Cancer Center recently reported some more simplified methods for personalized therapy. Moreover, (C) clinical genomic analyses *in silico* need more actionable clinical bioinformatics tools because most of clinical specimens are directly frozen at tumor tissue level after surgical removal. Clinical SNPs and genomic diagnoses require fairly reliable and cost-effective assay: genomics-based diagnostic tests have been greatly developed in direct therapeutic interventions. After a number of case-reports improving clinical practice with their cost-down, clinical genomic diagnosis test should be routinely developed to provide options for patients’ personalized therapy. System modeling and GES/SNP signature need more improvement: Recent development of cancer research has enabled physicians and scientists to use different genomic data such as GWAS, transcriptome from microarray and RNA-Seq, epigenetics profiles, microRNA profiles and non-coding DNA sequencing profiles. Now SNVs related with GWAS and transcriptome have been selected as personalized therapy though Encyclopedia of DNA Elements (ENCODE) have emerged in genomic analysis. In order to set up rational network for personalized therapy, in the future, ENCODE system biology should service in system biology and scoring quantitative network from all genomic profiles including transcriptome, SNPs, epigenetics and microRNA. On contrast, if more disease networks are established, local SNP detection combined with system modeling from different diseases’ network will be greatly improved for discovery of GES/SNP signature. Personalized targeted therapy

need discovering more targeted molecules and cost-effective.

Currently FDA approved targeted molecules are very limited for some molecule mechanism including tumor disease and very few other diseases and they are all very costly. Pharmaceutical companies need to develop more targeted molecules with cost-effective.

## Acknowledgment

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## Conflicts of Interest

The authors declare non-competing financial interests.

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