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

Received Date: July 16, 2015     Accepted Date: Aug 03, 2015 Published Date: Aug 06, 2015

Citation: Li, B. et al. Personalized Targeted Prevention and Therapy Relied on Detection of Global and local single Nucleotide Polymorphisms (2015) Int J Hematol and Therap 1(1): 1-8.

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[1]. 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”[2] and “targeted therapy”[3]. 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 change makes the patient susceptible to his disease or anticipates which medical prevention and treatments will be safe and effective for the patient[4]. 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[5]. 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 agents) 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[6]. 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.

Copy rights: ©2015 Li, B. This is an Open access article distributed under the terms of Creative Commons Attribution 4.0 International License.
Clinical Sampling for SNP And Genomic Analysis

Mixed-cells from clinical samples are greatly challenging for genetic diagnosis and genomic 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 report evidences, several sampling techniques have been applied for clinical SNP and genomic analysis. Clinical sampling includes clinical cells isolation consisting of flow-cytometric cell sorting (FACS)\(^8\), magnetic cell separation (MACS)\(^10\) and laser-captured micro-dissection (LCM)\(^11\) with downstream SNP and genomic analysis. 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)\(^8\), magnetic cell separation (MACS)\(^10\) and laser-captured micro-dissection (LCM)\(^11\) 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)\(^12\) and EpCAM for circulating tumor cells (CTCs)\(^13\). 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\(^14\). 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 for primary cells due to downstream genomic analysis\(^15\); (B) LCM can be used for automation system for high-throughput screening\(^16\).

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\(^17\). In 1994, 50 cases of primary tumor-cell culture were reported for drug sensitivity assay in our report\(^18\). 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\(^19\), 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%)\(^20\). 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 downstream 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\(^21\). 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[23-25]. 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[26]. 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[26]: 
- 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[28-31]. 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.
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[32]. 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 SNPs to GWAS to identify SNP related to 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)[33]. 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[34]. 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 using 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[35]. 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[36]. Networks with their knowledge are foundation of system modeling with intracellular components as nodes and their interactions as links within the system modeling[37]. 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[38]. 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[39]. The resulting graphs represent topology type-I of targeting genes to perform personalized therapy analysis as Figure 5B[40]. 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[41]; (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[42]; (3) Only SNPs data detected by designed SNP assay: a combination of disease-network and designed SNP discovery Table-2[43].

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            |         | Simple and easy to automate | lower throughput           | 0.6 per SNP/gene-otype |
| Sequen-phen | 10-300                   |                |         | Medium throughput   | Higher cost for equipment  | 0.2 per SNP/gene-otype |
| Illumina Golden-Gate | 384 — 3072 | 96 |         | Medium throughput   | Higher cost for equipment  | 0.2 per SNP/gene-otype |
| Captured-Seq | target-           | depending       |         | Higher throughput for a given | Higher cost equipment      | depending on sample size |
| Glob-al     | 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 |

Figure 5. A. Comparison of routine SNPs detection kits for PTPT. B. Example of GES with higher “Betweenness” and lower “connectivity”.
neGo and Pathway Studio are routinely used to discover small Genecards, Drug-bank and commercial software such as Ge- it can be used for both prevention and treatment. At present, lower toxic than any other therapy forms as we discussed above, personalization radiation. Moreover, personalized targeted therapy is personalized chemotherapy, personalized RNA therapy and per- for all therapeutic targeting of personalized therapy including tem modeling related with SNP signature can be directly used therapeutical targeting. Theoretically, genes uncovered by sys- tive archives although SNPs were largely reported to predict gene expression[44]. We have routinely added Qrt-PCR measurement as essential verification steps. Direct validation: Direct valida- tion-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 prolifera- tion using scoring analysis[49]. 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. Excit- ingly, after prediction and validation-mimic-assay by the python model, two groups’ drugs were mined and confirmed for the pa- tient, the patient achieved complete response after three months suggested drugs-treatment as Figure 8A,B[50]. (B): ex vivo mod- ule: As discussed above, clinical-cells cultures including prima- ry tumor-cell have been set up to assay drug sensitivity by Drs. Hamburger and Salmon. Now several protocols of primary tu- mor cell culture with their techniques are going to apply for val- idation system of personalized therapy[51]. Thus ex vivo module demonstrates a good system to validate suggested compounds for personalized therapy. (C): in vivo module: xenografts of hu- man 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[52]. Although it is cost- ly model, this model might become valuable model after reduc- ing 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[53]. 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 sup- port resistance mechanisms for the compounds. This model only can classifies therapeutic targets by genetic SNP determination in cancer cell lines.

**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/Wip1 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 sys- temic modeling related with SNP signature can be directly used for all therapeutic targeting of personalized therapy including personalized chemotherapy, personalized RNA therapy and personalization 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 Ge- neGo and Pathway Studio are routinely used to discover small molecules, Abs and drugs[44,45]. 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[47]. Ac- cording to published reports, here we list the validating meth- ods 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[44]. We have routinely added Qrt-PCR measurement as essential verification steps. Direct validation: Direct valida- tion-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[49]. 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. Excit- ingly, after prediction and validation-mimic-assay by the python model, two groups’ drugs were mined and confirmed for the pa- tient, the patient achieved complete response after three months suggested drugs-treatment as Figure 8A,B[50]. (B): ex vivo mod- ule: As discussed above, clinical-cells cultures including prima- ry tumor-cell have been set up to assay drug sensitivity by Drs. Hamburger and Salmon. Now several protocols of primary tu- mor cell culture with their techniques are going to apply for val- idation system of personalized therapy[51]. Thus ex vivo module demonstrates a good system to validate suggested compounds for personalized therapy. (C): in vivo module: xenografts of hu- man 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[52]. Although it is cost- ly model, this model might become valuable model after reduc- ing 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[53]. 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 sup- port 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.

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               |
|                            | Erlotinib        | EGFR cancer stem cell | NSCLC cancer stem cells |
|                            | Vemurafenib      | B-Raf enzyme Mek | melanoma melanoma   |
|                            | Temozolomide     | nTOR             | renal cancer        |
|                            | Everolimus       | nTOR             | renal cancer, three negative breast cancer |
|                            | Vemurafenib      | B-Raf enzyme Mek | melanoma melanoma   |
|                            | Cetuximab        | EGFR             | colon cancer and NSCLC |
|                            | Rituximab        | CD20 Her2/neu    | NHL breast cancer   |
|                            | Trastuzumab      | EGFR             | colon cancer and NSCLC |
|                            | Bevacizumab      | EGFR             | breast, brain, colon and NSCLC |

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 (ENCOD) have emerged in genomic analysis. In order to set up rational network for personalized therapy, 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

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       |
| In silico validation          | Bioinformatics modules | Bioinformatics supports | Mimic data          |
| Ex vivo validation            | Primary tumor cell or CSC culture | Objective response for different drugs | Difficult techniques |
| In vivo validation            | Xenografts of human cancer cells | Objective response for different drugs | Very costly and limiting time |
| In vitro confirmation         | Tumor cell lines cultures | Simple and easy for different drugs | Non-individual patient |

www.ommegaoonline.org

Int J Hematol and Therap  | volume 1: issue 1
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
Under the support of Dr. H. D. Preisler, we have set up these protocol to analyze clinical SNP and genomic diagnosis including single-cell genomic profiles of CD3, CD4 and CD8 from TIL and tumor cell from solid tumors. The work is supported by National Cancer Institute IRG-91-022-09, USA for Dr. Biaoru Li. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation.

Conflicts of Interest
The authors declare non-competing financial interests.

References
1. Rodríguez-Antona, C., Taron, M. Pharmacogenomic biomarkers for personalized cancer treatment. (2015) J Intern Med 277(2): 201-217.
2. Schilsky, R.L. Opinion: Personalized medicine in oncology: the future is now. (2010) Nature Reviews Drug Discovery 9: 363-366.
3. Yap, T.A., Bjerke, L., Clarke, P.A., et al. Drugging PI3K in cancer: refining targets and therapeutic strategies. (2015) Curr Opin Pharmacol 23: 98-107.
4. Hudson, T.J. Genome variation and personalized cancer medicine. (2013) J Intern Med 274(5): 440-50.
5. Yoshida, T., Ono, H., Kuchiba, A., et al. Genome-wide germline analyses on cancer susceptibility and GeMDBJ database: Gastric cancer as an example. (2010) Cancer Sci 101(7):1582-1589.
6. Pazin, M.J. Using the ENCODE Resource for Functional Annotation of Genetic Variants. (2015) Cold Spring Harb Protoc (6): 522-536.
7. Li, B. A strategy to identify genomic expression at single-cell level or a small number of cells. (2005) Journal of Biotechnology 8(1): 71-81.
8. Li, B. Clinical Genomic Analysis and Diagnosis --Genomic Analysis Ex Vivo, in Vitro and in Silico. (2012) Clinical Medicine and Diagnostics 2(4): 37-44.
9. Ormerod, G.M. Flow Cytometry: A practical approach. (2000) Oxford University Press.
10. Zhang, D.G., Jiang, A.G., Lu, H.Y., et al. Isolation, cultivation and identification of human lung adenocarcinoma stem cells. (2015) Onco Lett 9(1): 47-54.
11. Emmert-Buck, M.R., Bonner, R.F., Smith, P.D., et al. Laser capture microdissection. (1996) Science 274(5299): 998-1001.
12. Skvortsov, S., Debbage, P., Skvortsova, I. Proteomics of cancer stem cells. (2014) Int J Radiat Biol 90(8): 653-658.
13. Magbanua, M.J., Park, J.W. Isolation of circulating tumor cells by immunomagnetic enrichment and fluorescence-activated cell sorting (IE/FACS) for molecular profiling. (2013) Methods 64(2): 114-118.
14. Niyaz, Y., Stich, M., Sägmüller, B., et al. Noncontact laser microdissection and pressure catapulting: sample preparation for genomic, transcriptomic, and proteomic analysis. (2005) Methods Mol Biol 114: 1-24.
15. Steen, J., Morrison, J.A., Kulesa, P.M. Multi-position photoactivation and multi-time acquisition for large-scale cell tracing in avian embryos. (2010) Cold Spring Harb Protoc 16. Vandewoestyne, M., Van Hoofstat, D., Van Nieuwerburgh, F., et al. Automatic detection of spermatozoa for laser capture microdissection. (2009) Int J Legal Med (2): 169-175.
17. Hamburger, A.W., Salmon, S.E. Primary bioassay of human tumor stem cells. (1977) Science 197(4302): 461-463.
18. Li, B., Tong, S.Q., Zhang, X.H., et al. A new experimental and clinical approach of combining usage of highly active tumor-infiltrating lymphocytes and highly sensitive antitumor drugs for the advanced malignant tumor. (1994) Chin Med J (Engl) 107(11): 803-807.
19. Lähdesmäki, H., Shmuelovich, L., Dunmire, V., et al. In silico microdissection of microarray data from heterogeneous cell populations. (2005) BMC Bioinformatics 6: 54-58.
20. Geisler, T., Schaeffeler, E., Gawaz, M., et al. Genetic variation of platelet function and pharmacology: an update of current knowledge. (2013) Thromb Haemost 110(5): 876-887.
21. McBean, R.S., Hyland, C.A., Flower, R.L. Approaches to determination of a full profile of blood group genotypes: single nucleotide variant mapping and massively parallel sequencing. (2014) Comput Struct Biotechnol J 11(19): 147-151.
22. Cui, H., Dhroso, A., Johnson, N., et al. The variation game: Cracking complex genetic disorders with NGS and omics data. (2015) Meth- ods 1: 79-80C.
23. Hollegaard, M. V., Grauholm, J., Nielsen, R., et al. Archived neonatal dried blood spot samples can be used for accurate whole genome and exome-targeted next-generation sequencing.(2013) Mol Genet Metab 110(1-2): 65-72.
24. Koparir, A., Karatas, O.F., Atayoglu, A.T., et al Whole-exome se- quencing revealed two novel mutations in Usher syndrome. (2015) Gene 563(2): 215-219.
25. Li, W., Calder, R.B., Mar, J.C., et al. Single-cell transcriptom-omics reveals transcriptional exclusion of ENU-mutated alleles. (2015) Mutat Res 772: 55-62.
26. Pras, E., Krista, D., Shoshany, N., et al. Rare genetic variants in Tun- nesian Jewish patients suffering from age-related macular degeneration. (2015) J Med Genet 52(7): 484-92.
27. Kwok, P.Y., Chen, X. Detection of single nucleotide polymor- phisms. (2003) Curr Issues Mol Biol 5(2): 43-60.
28. Bjørheim, J., Ekstrom, P.O. Review of denaturation capillary electrophoresis in DNA variation analysis. (2005) Electrophoresis 26(13): 2520-2530.
29. Winchester, L., Yau, C., Ragoussis, J. Comparing CNV detection methods for SNP arrays. (2009) Brief FuncGenomic Proteomic 8(5): 353-66.
30. Witherden, E.A., Kunde, D., Tristram, S.G. An evaluation of SNP- based PCR methods for the detection of β-lactamase-negative ampicil- lin-resistant Haemophilus influenzae. (2012) J Infect Chemother 18(4): 451-455.
31. Knez, K., Spasic, D., Janssen, K.P., et al. Emerging technologies for hybridization based single nucleotide polymorphism detection. (2014) Analyst 139(2): 353-370.
32. Katara, P. Single nucleotide polymorphism and its dynamics for pharmacogenomics. (2014) Interdiscip Sci 6(2): 85-92.
33. Allman, R.B., Bromberg, Y. Collective judgment predicts disease-associated single nucleotide variants. (2013)BMC Genomics 3: S2.
34. Quetglas, I.M., Moeini, A., Pinyol, R. et al. Integration of genomic information in the clinical management of HCC. (2014) Best Pract Res Clin Gastroenterol 28(5): 831-842.
35. Liu, G., Qin, Y., Li, Z., et al. Development of highly efficient, low-cost lignocellulolytic enzyme systems in the post-genomic era. (2013) Biotechnol Adv 31(6): 962-975.
36. Novosyadlyy, R., Leroith, D. Insulin-like growth factors and insu- lin: at the crossroad between tumor development and longevity. (2012) J Gerontol A Biol Sci Med Sci 67(6): 640-51.
37. Poult, S.R., Promislov, D.E.L., Phillips, P.C. Network thinking in network science. (2012) Curr Opin Pharmacol 23: 98-107.
38. Zhao, J.H. Pedigree-drawing with R and graphviz. (2006) Bioinfor- matics 22: 98-107.
39. Hu, G., Zhou, J., Yan, W., et al. The topology and dynamics of pro-
tein complexes: insights from intra-molecular network theory. (2013) Curr Protein Pept Sci 14(2): 121-132.
40. Li, B., Senzer, N., Rao, D.D., et al. Bioinformatics Approach to Individual Cancer Target Identification. (2008) 11th Annual Meeting of the American Society of Gene Therapy C8: 451004.
41. Soderlund, C.A., Nelson, W.M., Goff, S.A., Allele Workbench: transcriptome pipeline and interactive graphics for allele-specific expression. (2014) PLoS One 9(12): e115740.
42. Koh, Y., Kim, D., Jung, W.J., et al. Revealing Genomic Profile That Underlies Tropism of Myeloma Cells Using Whole Exome Sequencing. (2015) Int J Genomics. 2015: 675379.
43. Fenger, M., Linneberg, A., Jepepesen, J. Network-based analysis of the sphingolipid metabolism in hypertension. (2015) Front Genet 6: 84.
44. Wishart, D.S., Knox, C., Guo, A.C., et al. DrugBank: a knowledge-base for drugs, drug actions and drug targets. (2008) Nucleic Acids Res 36 (Database issue): D901-906.
45. Harel, A., Dalah, I., Pietrokovski, S., et al. Omics data management and annotation. (2011) Methods Mol Biol 719: 71-96.
46. Vari, S., Pilotto, S., Maugeri-Saccà, M., et al. Advances towards the design and development of personalized non-small-cell lung cancer drug therapy. (2013) Expert Opin Drug Discov 8(11): 1381-1397.
47. Lossos, I.S., Czerwinski, D.K., Alizadeh, A.A., et al. Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. (2004) N Engl J Med 350(18): 1828–1837.
48. Zheng, J., Zhang, D., Przytycki, P.F., et al. SimBoolNet—a Cytoscape plugin for dynamic simulation of signaling networks. (2010) Bioinformatics 26(1): 141-142.
49. Hu, H.L., Zhang, Q.H., Li, S., et al. A Therapeutic Targeting Identification from Microarray Data and Quantitative Network Analysis. (2015) The Open Access Journal of Science and Technology 3: 1-10.
50. Riddick, G., Song, H., Holbeck, SL., et al. An in silico screen links gene expression signatures to drug response in glioblastoma stem cells. (2014) Pharmacogenomics J 15(4): 347-353.
51. Dairkee, S.H., Ji, Y.G., Ben, Y. A molecular ‘signature’ of primary breast cancer cultures; patterns. (2004) BMC Genomics 5: 47.
52. Patsialou, A., Wang, Y.R., Lin, J. Selective gene-expression profiling of migratory tumor cells in vivo predicts clinical outcome in breast cancer patients. (2012) Breast Cancer Res 14(5): R139.
53. Zheng, Y., Zhou, J., Tong, Y. Gene signatures of drug resistance predict patient survival in colorectal cancer. (2015) The Pharmacogenomics Journal 15: 135-143.