Where cancer genomics should go next: a clinician’s perspective

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Large-scale, genomic studies of specific tumors such as The Cancer Genome Atlas have provided a better understanding of the alterations of pathways involved in the development of solid tumors including glioblastoma, breast cancer, ovarian and endometrial cancers, colon cancer and lung squamous cell carcinoma. This tremendous effort of the scientific community has confirmed the view that cancer actually represents a wide variety of diseases originating from different organs. These studies showed that TP53 and PI3KCA are the two most mutated genes in all types of cancers and that 30–70% of all solid tumors harbor potentially ‘actionable’ mutations that can be exploited for patient stratification or treatment optimization. Translation of this huge oncogenomic data set to clinical application in personalized medicine programs is now the main challenge for the future. The gap between our basic knowledge and clinical application is still wide. Closing the gap will require translational personalized trials, which may initiate a radical change in our routine clinical practice in oncology.

INTRODUCTION

During the past few decades, our approach to prevention, diagnosis and treatment of cancer has radically shifted from organ-based to morphology-based and most recently, to genetics-based. Personalized or precision medicine (tailoring a treatment for a patient’s particular disease at a precise time point) is being performed everyday in the clinical setting at different levels. For instance, prevention involves bilateral mastectomy and adnexectomy for patients with BRCA risk variants (1). For patient stratification, mutations in KRAS were demonstrated to be prognostic and predictive in Non-Small Cell Lung Cancer (NSCLC) (2). In some cases, therapeutic strategies are guided by gene overexpression such as herceptin treatment in Her2+ breast cancer patients (3).

Whether oncogenomics will be used in a clinical setting is no longer the question (4,5). The main challenge going forward will be to deal with the great quantity of genomic information that will be made available to clinicians and patients by the latest high-throughput sequencing platforms. Indeed, easy access to tumor genetic information will drive customization of patients’ care not only at the diagnosis stage, but also during treatment and possibly, most importantly, in the case of cancer recurrence. Our deeper understanding of cancer genomes has led to new landscapes such as genomic prognostic signatures (sometimes many different ones for a particular cancer) (6), radical inter-patients and intra-patient tumor heterogeneity and tumor genomic, genetic and epigenetic evolution during treatment (7–10). The importance of all these findings in the clinical setting needs to be comprehensively investigated. While the clinical community starts to grasp the enormous opportunity offered by our ability to obtain, in a clinically relevant timeframe, a full genomic portrait of a tumor, many challenges need to be overcome to translate all our knowledge in the clinical setting.

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In this review, we focus on the clinical challenges raised by the advent of the oncogenomics area. Our intent is not to review all the genomic advances but to provide a clinical perspective on the future roles of genomics in cancer care. While the effort of several international consortia has led to deep understanding of many tumor genomics, the pace of translation of our oncogenomics knowledge to the clinic is still quite slow with only a few examples. At this point, there is on one side the data generated through the use of high-throughput platforms and on the other side a number of clinical trials that have implemented a personalized approach to cancer care. Bridging the gap requires an understanding of how to integrate the knowledge that has been collected in a clinical setting. Here, we describe the principles that have led to the first application of oncogenomics in clinical oncology as they illustrate the path toward translation. We will summarize a selection of major tumor-specific findings from the large cancer oncogenomic consortia and describe the clinical trials that have been conducted to date and that are illustrating how to translate genomic data into clinical practice.

TRANSLATION OF ONCOGENOMICS DATA RELIES ON THE UNDERSTANDING OF SIGNAL TRANSDUCTION AND THE BIOLOGICAL CONSEQUENCES OF THE GENOMIC ALTERATIONS

The era of genomic medicine in oncology started in the 1980s when the relationship between karyotype abnormalities and patients’ disease was identified, thereby allowing better patient stratification (1). Soon this approach became common in both leukemia as well as in solid tumors treatment (11–13). The next step was to define the role of oncogenes and proto-oncogenes in tumor formation and progression (14,15). Finally, by establishing the mutational profile of different tumors, it became clear that there is an accumulation of a high number of mutations within a tumor, which led to the concept of driver and passenger mutations (14,15).

Today high-throughput genome sequencing approaches allow us to investigate a tumor at multiple levels: (i) a mutational profile and large chromosomal abnormalities can be obtained by exome sequencing, (ii) gene expression abnormalities can be identified using RNA sequencing, and (iii) epigenetic deregulation can be measured through bisulfite and/or chromatin immunoprecipitation-sequencing technologies. However, we have not been able to concomitantly develop the experimental framework that would allow the robust functional and therapeutic validation of the multiple potentially identifiable targets. Indeed, studies with cell lines and in vivo assays are limited by our ability to reproduce multiple major aspect of tumor biology: global genetic context, microenvironment interaction and therapeutic regimens (16–20). All these different elements can modify the functional consequences of a genomic abnormality and the effect of its medical targeting.

Once a driver gene has been clearly identified, the genetic context plays a major role. Indeed, recent mutational analysis identified BRAF mutations as a major driver in melanoma, and some cases were treated with great success by BRAF inhibition (21,22). Translation of BRAF inhibitors to the clinic required only 8 years compared with the previously required 13 years for translation of the Her2 blocking approach. This shortening of the translation time demonstrates the efficiency of the oncogenomic-based approach (23). However, in colon cancer, the BRAF mutational profile predicted resistance to anti-EGFR treatment (24). Moreover, BRAF inhibition with a V600E mutation causes a rapid feedback activation of EGFR, supporting continued proliferation in the presence of BRAF inhibition in patients with this genetic variant (25). The BRAF paradigm is one of the most interesting in terms of oncogenomic-personalized medicine. Indeed, if we carefully analyze the pathway toward clinical application, we can identify three major steps:

(i) Discovery of a driver mutation with functional consequences in a particular disease:
- Inhibition of the protein that carries the mutation;
- Patient stratification for treatment;
- Proof of principle of efficiency as demonstrated by improved progression free and overall survival.

(ii) Application of the inhibition based on oncogenic studies to other diseases:
- Partial success;
- Basic studies defining an alternative way of resistance in a different genetic/cell type context.

(iii) New patient stratification in a new disease.

‘Bref’ personalized medicine really requires true personalization. In other words, the identification and targeting of a driver mutation in a tumor type does not mean efficiency in other tumor types. We need to optimize our therapeutic regimens considering parameters such as tissue specificity, genetic environment and tumor microenvironment.

Multiple new-targeted therapeutics will likely go through the same developmental pathway. For instance, Imatinib (Gleevec®), a drug initially developed to treat Bcr-Abl chronic myeloid leukemia, is now being used in cKit+ gastro-intestinal stromal tumor and melanoma (26–30). In parallel, systematic ancillary translational studies in clinical trial are resulting in great therapeutic consequences. In many clinical trials, gene expression or mutational profile are being concomitantly assessed and give us insight into patients’ stratification and treatment efficiency. For example, in a clinical trial assessing EGFR inhibitor in NSCLC, targeted sequencing indicated more efficiency of the inhibitor in patients with kras mutation than without, resulting in better patient stratification (8 out of 9 responders vs none of the 7 non-responders) (2). Although genomics can inform clinical treatment, the approach of integrating transcriptomic and mutational profiling to clinical trials will lead to clinically informed genomics (discussed below). The feedback of the clinic to genomic analysis is an integrated part of the research should speed the translational process.

Overall, at this point, most of the targeted therapies did not emerge from large oncogenomics studies but through a deep understanding of specific pathways in defined tumor types. Most of the data reviewed so far have emerged from studies of a single or a limited number of mutation(s). However, the development of new technological platforms has radically modified our view of the tumor genome. Within the last 4 years, many international studies/consortia used a comprehensive approach...
to determine a multi-layer tumor profile. The data obtained and described below now needs to mature to be integrated in our clinical practice.

WHAT HAVE WE LEARNED ABOUT DIFFERENT TUMORS THROUGH LARGE ONCOGENOMIC STUDIES?

The large genomics consortium have identified recurrent point mutations, translocations and potentially new therapeutic targets in more than 20 (the Cancer Genome Atlas) and 50 (International Cancer Genomics Consortium) cancer subtypes (31,32).

In the following paragraph, we highlight some of the main findings uncovered by a high sequencing throughput approach to a few selected tumor types.

Glioblastoma (GBM)

Multi-layer characterization of glioblastoma was the first study published by The Cancer Genome Atlas (TCGA) (33). It demonstrated the ability to comprehensively assess a tumor type’s oncogenic landscape through an integrated approach. Importation of mutations in genes such as TP53 (37% of all the tumors sequenced) and NF1 (14% of all the tumors) was confirmed. EGFR alterations [focal amplification, defined as 3 Mb or smaller in size (34)], point mutations were observed in 41 of the 91 sequenced samples. Finally, mutations were found in ERBB2 and PI3KCA complex. The study also pointed out epigenetic abnormalities such as MGMT promoter methylation in 19 out of 91 samples. Such abnormalities were associated with a hypermutated profile in the treated patients. Finally, by integrating all data sets, the authors could define three core gene sets/pathways: receptor tyrosine kinase (RTK) signaling, the p53 and the RB tumor suppressor pathways. Of the 206 samples, 66, 70 and 59% had somatic alterations of the RB, TP53 and RTK pathways, respectively, at the CNV level. The authors defined a gene expression-based molecular classification of glioblastoma (GBM) into proneural, neural, classical and mesenchymal subtypes (33). The classical, mesenchymal and proneural subtypes harbored aberrations in gene expression of EGFR, NF1 and PDGFRA/IDH1, respectively. Finally, their study showed that the response to aggressive therapy differed by subtype, with the greatest benefit in the classical subtype and no benefit in the proneural subtype (33).

Ovarian cancers

The second report of the TCGA group focused on 489 patients with papillary serous ovarian cancer (35). The TCGA determined that TP53 mutations were present in almost all tumors (96%). Using a background mutation rate, they found a low but significant rate of mutations in nine further genes, including NF1, BRCA1, BRCA2, RB1 and CDK12. They showed that ovarian carcinomas harbored a high number of CNVs (113 focal CNVs) and promoter methylation (168 genes overall were epigenetically silenced), concordant with other reports. An integrated approach allowed for the identification of four ovarian cancer transcriptional subtypes, three microRNA subtypes, four promoter methylation subtypes and a transcriptional signature associated with survival duration. Different subtypes with impact on prognosis were defined later using the same data set (36–39) suggesting that similar oncogenomics information can lead to different tumor signatures depending data used and the statistical approach.

Colon and rectal cancer

The TCGA group included 276 samples in this analysis (40). They identified 16% of colorectal carcinomas as being hypermutated with three-quarters harboring high microsatellite instability (hypermutation and MLH1 silencing) and one-quarter had somatic mismatch-repair gene and polymerase ε (POLE) mutations. In total, 24 genes were significantly mutated above the background mutational rate (40). Some were described previously (APC, TP53, SMAD4, PIK3CA and KRAS mutation), while others such as ARID1A, SOX9 and FAM123B were newly defined through in the TCGA analysis. The recurrent CNVs included amplifications of ERBB2 and IGF2. The WNT, MAPK, PI3K, TGF-β and TP53 pathways were frequently altered. An integrated analytical tool: PARADIGM (41) was used to uncover new molecular aberrations such as dysregulation of the MYC pathway in almost all tumors (40).

Lung squamous cell carcinoma

One hundred and seventy-eight lung SqCCs were profiled (42). These tumors displayed a very high degree of alterations with a mean of 360 exonic mutations, 165 genomic rearrangements and 323 segments of copy number alteration per tumor. Eighteen genes presented recurrent mutations. Almost all patients had a TP53 mutation. Other genes involved were CDKN2A, PTEN, PIK3CA, KEAP1, ML2, HLA-A, NFE2L2, NOTCH1 and RB1. The pathways altered were NFE2L2/KEAP1 in 34%, squamous differentiation genes in 44%, PI3K/AKT in 47% and CDKN2A/RB1 in 72% of tumors. mRNA profiling retrieved four subtypes designated as classical (36%), basal (25%), secretory (24%) and primitive (15%). The miRNA and methylation profile were associated with the different subtypes described. Finally, the authors could define therapeutic targets for most of the tumors (42). Amplification of EGFR resulting in increased sensitivity to erlotinib and gefitinib was found in 7% of cases. Ninety-six percent of tumors contained one or more mutations in tyrosine kinases, serine/threonine kinases, PI3K catalytic and regulatory subunits, nuclear hormone receptors, G protein-coupled receptors, proteases and tyrosine phosphatase. Among them, therapeutic target analysis identified 64% of potentially targetable genes, including ERBBs, FGFRs and JAKs.

Breast cancer

The comprehensive analysis of 510 breast tumors allowed the retrieval of the four subtypes described previously (43). The mutational landscape contained the classical long-established genes PIK3CA, PTEN, AKT1, TP53, GATA3, CDH1, RB1, MLL3, MAP3K1 and CDKN1B beside a number of novel significantly mutated genes above the background mutational rate (SMGs) such as TBPX, RUNX1, CBFB, AFF2, PIK3R1, PTPN22, PTPRD, NF1, SF3B1 and CCND3. Luminal A and luminal B
tumors displayed more diverse mutations than basal-like and HER2+ subtypes (43). Luminal A tumor had MAP3K and MOP2K4 mutations. Luminal B mutational profile was quite diverse; however, TP53 and PI3KCA were the most frequent mutations (29%). Basal-like cancers had a high rate of TP53 mutations (80%) and did not share any mutation with the luminal subtype. Finally, the HER2+ subtype had a hybrid pattern with a high frequency of TP53 (72%) and PIK3CA (39%) and lower rate of other mutations (43).

**Endometrial cancer**

The most interesting finding in endometrial cancer was the ability of the authors to stratify patients into different subgroups (44). At one extreme, the group is hypermutated (18*10^-6 mutations per Mb) with quite good prognosis, and at the other extreme, patients that harbor a single TP53 mutation (90% of patients in this subgroup) together with a large number of CNVs have quite poor prognosis. While these classifications match the pathology classification to a certain extent, we could potentially translate these findings in the clinic quite easily and establish a genomic-based therapeutic protocol that could directly impact patients’ treatment. Indeed most probably, patients with a hypermutated tumor do not need extensive surgery, including lymph node removal, and their genomic profiling could avoid extensive surgical morbidities. Interestingly, the poor prognosis endometrial cancers clustered with ovarian papillary serous cancer and breast basal-like tumors (Focal SCNA patterns, TP53 mutational profile) advocating once more for a biological rather than organ-based approach (44).

**Global analysis**

The tremendous effort of the scientific community confirmed that cancer represents a wide variety of disease originating from different organs. The role of many specific molecules or pathways has been confirmed in multiple subtypes with tumor-type-specific function. The need for new and more efficient therapeutics requires a better global understanding of tumor-type-specific function. The need for new and more efficient therapeutics requires a better global understanding of pathways has been confirmed in multiple subtypes with mutational profile as well as to clinical outcomes; (c) Large chromosomal abnormalities (CNVs) play a major role in tumor biology; (d) Clustering across organs allow a biology driven approach that considers important cellular pathways rather than simple tumor morphology, etc.

Overall, these selected tumors illustrate the following aspects:

1. We have a comprehensive profile of the most common tumors.
   a. The mutational profile demonstrate few driver mutations shared across tumor subtypes;
   b. Gene expression and/or miRNA allow stratification of tumors in different subtypes that are often correlated with mutational profile as well as to clinical outcomes;
   c. Large chromosomal abnormalities (CNVs) play a major role in tumor biology;
   d. Clustering across organs allow a biology driven approach that considers important cellular pathways rather than simple tumor morphology, etc.

2. We can generate the data and obtain the results in a clinically adapted timeframe.

3. For the first time in oncology history, the drugs that could potentially inhibit the oncogetic pathways are available.

**ONCOGENOMICS IN CLINICAL PRACTICE**

Clinical and genomic reports suggest that as many as 30–70% solid tumors harbor potentially ‘actionable’ mutations or gene variants that can be used for patients stratification or treatment optimization (45,48). Hence, many institutions in the world have initiated large personalized medicine programs in oncology. The MD Anderson initiated such a program in the context of early clinical trials matching drugs with tumor molecular aberrations in 2007 and reported the results in 2012 (49). A total of 1114 patients with advanced metastatic disease were enrolled, of which 40.2% (n = 460) had one or more aberration tested by PCR-based sequencing technology (49); 175 patients were treated with a matched targeted therapy directed against their matched aberration and displayed higher overall response rate (27 versus. 5%; P < 0.0001), longer time-to-treatment failure (TTF; median, 5.2 versus. 2.2 months; P < 0.0001) and longer survival (median, 13.4 versus. 9.0 months; P=0.017) than 116 treated with unmatched therapies. Targeted therapy was an independent factor for TTF and predicting response in patients with one molecular aberration, while the overall response rate can be considered low in a matched population. Some aspects of the study were promising. For example, patients with a BRAF mutation displayed a response rate of 37% in the matched therapy group compared with 0% with non-matched therapy (P = 0.004) (49). There was no significant difference
in response in patients with more than one aberration, regardless of matched or unmatched therapies. This shows our ability to improve treatment on single aberrations while highlighting our lack of understanding of the interaction between multiple aberrations. Overall, the results of this study can be considered positive as patients represent a heterogeneous population with multiple prior treatment regimens. Interestingly, the patients and physician eagerness to participate in such a personalized medicine trial seem to be high with a better than expected rate of inclusion.

Similar design was used in the MOSCATO-01 trial (Molecular profiling in Cancer for Treatment Optimization). Preliminary results were disclosed last year at the ASCO meeting (50). This translational trial conducted at the Gustave Roussy cancer center included patients with treatment-resistant progressive metastatic neoplasia with at least a lesion accessible to biopsy and molecular profiling. Comparative genomic hybridization as well as whole genome sequencing was used to guide targeted therapy. Progression free survival (PFS) using therapy based on genomic alteration was then compared with the PFS for the most recent therapy on which the patient had experienced progression (PFS ratio) (50). Among the 129 patients enrolled, 111 (86%) could undergo a biopsy demonstrating a high rate of acceptance and technical feasibility, which could likely be increased using liquid biopsy modalities (circulating tumor cells and circulating tumor DNA). An actionable target was identified in 40% of the patients \( (n = 52) \). Among them, 52% \( (n = 25) \) were treated accordingly; 20% \( (n = 5) \) had a partial response, 56% \( (n = 14) \) had stable disease and 12% \( (n = 3) \) had progressive disease. The trial is continuing.

The set-up of these trials has helped inform the clinical and scientific community about the hurdles to overcome. Indeed, having an actionable target is not enough. Genetic context, tumor heterogeneity and tumor evolution under treatment play a major role in the therapeutic response. New studies should help better establish this new framework for genomic informed oncology. Indeed, specialized tumor boards are being set up with new expertise such as bio-informatics to better integrate the necessary tools for interpretation and implementation: we need continued creativity and cross-discipline expertise to build the necessary infrastructure to support these efforts (51).

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CONCLUSION

There is no doubt that we have entered a new era of cancer treatment, particularly from a clinical point of view. Within few years, we have shifted from treating breast cancer as an amorphous entity into treating it with some level of specificity, such as basal-like breast cancer. We have shifted from treating melanoma as a single entity, to specifically treating BRAF-mutated melanoma. While one can only be enthusiastic when looking at the tremendous quantity of data now being collected, there is still a long path to clinically apply our broad knowledge of tumor biology. The gap between our basic knowledge and clinical application is still wide and is only slowly being filled in through the implementation of well-designed personalized medicine trials (Fig. 1). Our community now needs tremendous creativity and cross-discipline expertise to build the necessary tools for interpretation and implementation: we need continued large, high-resolution, clinical-genomic data sets; we need better and earlier access to drugs; we need to create new expertise such as bio-informatics to better integrate the knowledge with the clinicians. One goal is to gather expertise on tumor genomes and biology rather than just an organ (classically tumor boards focus on particular organs rather than tumor biology). In this context, the positive results of the SAFIR01 trial addressing the role of matched therapies in advanced metastatic breast cancer were a perfect example of such a set-up (51).

In a single year, this multicenter trial enrolled 423 patients for which CGH array and Sanger sequencing identified a targetable genomic alteration in 195 (46%) patients. These were most frequently in \( PIK3CA \) (25%), \( CCND1 \) (19%) and \( FGFR1 \) (13%). Thirty-nine percent of the patients with test results available presented a targetable mutation. Therapies could be matched in 13% of the overall study population. Among 43 patients finally treated, 4 (9%) presented an objective response [according to the Response Evaluation Criteria in Solid Tumors (RECIST)], and 9 (21%) had stable disease for more than 16 weeks. The authors discussed the optimization of drug access and the up-scaling of such trials to obtain robust data sets that will lead to optimization of personalization (51). Finally, we wish to point out that a major limitation in these studies is the fact that heavily pre-treated patients are included. We, therefore, need to develop a better framework to include patients earlier where tumor drift and chemotherapy selection as well as tumor heterogeneity are less advanced.

Finally, an optimal way to deal with these aspects might be to use sequencing as a dynamic tool, and take advantage of patients that can be called outliers (surprisingly good response or poor response to therapies) (52). Such strategies used in bladder cancer demonstrated that whole genome sequencing could be performed in a clinical setting. This single patient approach can be useful to uncover new mechanisms of response or resistance. An example of this is the case of mTORC1-directed therapies which may be most effective in cancer patients whose tumors harbor TSC1 somatic mutations (53).
as onco-bio-informaticians and genome experts; our translational tumor boards will have to integrate onconcogenomics data into clinical situations (primary versus metastasis, recurrence and previous treatment). A few years ago at an AACR keynote lecture, B. Vogelstein declared that if we continued to think about cancer the way we did we would fail in our aim for cure. He was absolutely right and the change of direction has already started.

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