Translational Proteomics: What Can You Do for True Patients?

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Matching the right medical strategy to the right patient is the key for modern clinical oncology. To this aim, we have many delicate drugs designed to target in elegant ways critical proteins identified in cancer cells. However, clinical oncologists and multidisciplinary groups devoted to treating patients in an integrative fashion have histology and an TNM staging system as the most relevant biomarkers to decide therapeutic approaches for our patients. In addition, the most used drugs are classical chemotherapeutic compounds such as cisplatin, epirubicin, irinotecan, oxaliplatin, and so on. Thus, new targeted therapies, surgery, radiotherapy, and chemotherapy will live together causing a duality for the immediate future. We will try to delineate unmet needs for clinical oncologists that would add value for cancer proteomics in terms of true patients.

Keywords: translational proteomics • biomarker • cancer

Personalized and individualized medicine is based on the principle of identifying the right drug for the right patient before the administration of the drug. For clinical scientists to match treatment strategy to both the tumor features and the patient, demographics must be addressed by specific, reproducible, and scientifically robust biomarkers approved by regulatory Agencies (EMEA for European Union and FDA for USA). In this regard, more than 170 000 manuscripts published in the last 30 years reflect the interest we all have in biomarkers. Every week of every month, enthusiastic readers observe hundreds, thousand of biomarkers published in peer-reviewed journals. These potential biomarkers are devoted to predict the biological behavior of a sarcoma, calculate the probability to respond to cisplatin of nonsmall cell lung cancer, or foresee the need of adjuvant chemotherapy for a woman diagnosed with early breast cancer. Unfortunately, less than 0.1% of every published biomarker will be of clinical utility. Therefore, every day, every week, and every month we administer chemotherapy, radiotherapy, or very expensive targeted therapies to modify the natural history of cancer. We have to treat patients that a surgeon probably has cured only with a lumpectomy, because we do not know if she is really cured. We administer cisplatin, taxanes, vinca alkaloids, and so on to patients that will probably never respond to these drugs but we will know this intrinsic resistance only after 6 weeks of therapy. We avoid the use of many drugs that in a clinical trial evidenced a response rate about 4% because we are unable to identify this subset of patients. What does it mean? We have fewer biomarkers than fingers on our hands. And close to 90% of our patients with disseminated disease will die and 2 out of 5 of our patients diagnosed with localized disease will also die. Currently, more than 800 anticancer drugs are on the way and huge efforts are being made to identify new targets to introduce these novel drugs to treat cancer. Probably, we have deciphered a considerable amount of the entire human genome and many groups have collaborated to get the genome for lung cancer, glio-blastoma multiforme, pancreatic cancer, and others. But we do not know if we have to use cisplatin or paclitaxel, drugs designed more than two decades ago. Why? What can we do for our current patients? For our next-decade patients, preventive medicine will help them. What now? In this paper we will try to delineate the requirements we urgently need to improve our clinical practice that will help us to trigger the next technology revolution that our oncology hospitals must undergo in future years.

Predictive Proteomics: a Link among Patients, Prognosis, and Surgery

The probability of recurrence for a tumor after a curative surgery is a major concern for patients and physicians. Patients put their own lives at risk in complicated surgeries, and many of them will be treated with adjuvant chemotherapy, radiotherapy, or both. All of them will be exposed to potential toxicity, but only a few will benefit. In this regard, all of our clinical efforts are directed to balance these risks with the natural history of untreated cancer, attempting to increase overall survival (OS) and disease-free survival (DFS) for an entire population. We usually express these variables as medians or Hazard Ratio for the probability to die (OS) or recur (DFS) after a curative surgery. This information is critical because when our patients ask for prognosis, we usually
comment data based on median OS or DFS from clinical trials (50% of the entire population will die and 50% will be alive at X months).

Currently, the most widely (and accurate) prognostic factor used is TNM classification (T for Tumor, N for Node, and M for metastases) that is identified by macroscopic examination of fresh tumor, light microscopic evaluation of paraffin embedded slides, and PET-CT. For example, the local growth of primary lung cancer, its relations with surrounding tissues, and the status of regional lymph nodes are critical factors for staging patients diagnosed with a localized nonsmall cell lung cancer. Patients with no regional lymph nodes invaded by a nonsmall cell lung cancer have a 60% chance to survive 60 months, while those that have invasion on nodes that lie within the mediastinal pleural envelope will die within 60 months with an 80% of probability. Nowadays, adjuvant chemotherapy based on cisplatin doublets may reduce this probability by 10% at 5 years. For breast cancer patients, our results in terms of prognosis at early stages have been improved in recent years with the onset of commercially available gene profiles6,7 that help clinicians to decide adjuvant chemotherapy for T1N0M0 breast cancer patients. Similarly, an 18-gene signature has been recently suggested to calculate prognosis for colon cancer patients8 and many others are under development for several tumors. So, is genomic profiling of DNA or RNA extracted from tumoral samples from oncologic patients the technological approach that solves our clinical problems in terms of prediction? We do not know, but the clinical fact is that the cancer and the patient are biologically dynamic. From a simplistic point of view, we observe everyday how the mutational alterations of K-Ras9 or c-Kit10 and the expression of c-erbB2 or estrogen receptor11 have modified their status when primary and metastatic sites are compared. Additionally, recurrence is associated to distant imperceptible disease, resistant to adjuvant chemo or radiotherapy, from which we do not have a sample to profile. Finally, the concordance among mRNA expression and protein function is not linear, impairing the design of new drugs based on mRNA expression. So, is the genomic profiling from primary tumor enough to decide the treatment for microscopic, undetectable disease? Do we have an alternative biological sample that is easy to collect and that does not alter our daily practice of maintaining our patients comfort? Does this sample enhance genomic profile information from primary tumors that dynamically updates our information about the oncological status of a specific patient? Please, do not forget the critical question: will this patient recur?

Resolving the Puzzle of Chemotherapy, Radiotherapy, Targeted Therapies, or Both: Proteomics to Decide

Currently, our biomarkers to decide chemotherapy are histology and Computed Tomography (CT) in combination with demographic data that our assistants and residents collect at 7:00 a.m. Surprisingly, a technology designed four centuries ago to observe textile fibers in combination with the primary localization of the tumor decide if we use a given chemotherapy schedule for lung, breast, and colon cancer. These mirror the enormous gap among the technology we use to decide chemotherapy, radiotherapy, or targeted therapies and the technology needed to design a drug effective against cancer. Is this gap responsible for the lack of success of many new drugs in clinical trials? New therapies for cancer patients are accepted on the basis of increased efficacy or decreased toxicity evidenced in phase III, randomized controlled trials. In spite of hopeful results in earlier phase II studies, almost all treatment regimens do not show statistically significant benefits when tested in powered trials that compare standard therapy with experimental arms. However, clinicians that recruit patients for clinical trials observe how a few patients benefit from the experimental therapy. Frequently, we also observe how this trial provides negative/neutral results but it is our conviction that the drug works well in a group of patients. In this regard, a small group of patients (e.g., less than 5%) are not enough to have an impact on the statistical difference between two arms of therapy. Finally, the investigator and the interested reader may conclude that this experimental approach has no benefit. Let me show an example: gefitinib is an inhibitor for the tyrosine kinase activity that resides in an intracellular domain of EGFR. It was designed to target a wild type EGFR and showed exciting efficacy in phase I and II trials. When it was evaluated in phase III, it was combined with chemotherapy, both the standard schedule in Europe, Japan, and the United States. And the results were a complete disaster.12,13 However, oncologists that recruited patients for this trial had observed that women, never smokers, got an excellent response, both clinical and radiological. And many of these patients that took part in first trials still take gefitinib. A few years later, Kobayashi et al.14 evidenced an unbelievable activity of gefitinib on EGFR mutated on the tyrosine kinase domain. Then a few groups identified that those patients that got an excellent response to the drug were patients that had a mutated EGFR, and those patients were mainly women and never-smokers. Ten years later, gefitinib is approved for patients diagnosed with nonsmall cell lung cancer with mutated EGFR,15 but thousands of patients have not received this drug during those ten years. Interestingly, gefitinib was designed against wild type EGFR16 and was tested in an unselected population of nonsmall cell lung cancer. Erlotinib is a similar drug, designed against a wild type receptor and approved for second line patients as well as for naive patients that are unable to receive cytotoxic chemotherapy. Pivotal clinical trials evidenced a small but clinically relevant benefit for unselected patients17 that were counteracted by the enormous gain observed in those patients that harbor a mutated EGFR on their tumors.18 However, we observe how a small group of patients without EGFR mutations improve their quality of life, prolong their survival, and reduce tumors size when they are treated with erlotinib. So, if we decide to administer gefitinib or erlotinib exclusively for EGFR mutated patients, wtEGFR patients with an unknown mechanism of response to these drugs will never receive an acceptable therapy. On the opposite, if we test gefitinib or erlotinib in unselected populations of nonsmall cell lung cancer patients, we will overpower the trial and may erroneously conclude that the drug does not work. The fact is that all our trials are underpowered of patients with true possibilities of benefit.

Far beyond this scenario, classical chemotherapy compounds are still the cornerstone for the treatment of our patients. In gastric cancer patients, chemotherapy schedules provide a range of survival times from 5 to 25 months. Taxanes or anthracyclines in combination with cisplatin and 5-Fluorouracil are the most widely used schedules in stage IV (disseminated) patients. Docetaxel targets beta-tubulin at the microtubules, impairing their dynamic properties critical for mitosis, intracellular trafficking and cellular movements. If we look for the tip of the iceberg, we would evaluate the tubulin sequence or the tubulin isotype. But if we want to calculate the size, the composition, and the origin of the entire iceberg,
what would you do? Please export this question to all the tumors and patients because we are trying to solve the traffic problems of Madrid or NYC looking at the engine of the F1 of Fernando Alonso.

As described above, cancer is a dynamic disease with specific space-temporal expression of myriad of genes that codifies for hundreds of proteins with impaired (or not) functions. This expression varies along the therapy to resist cancer therapy as well as across the different areas of metastases but also within the same area where different metastases coexist. Additionally, the HapMap project showed that variability among individuals is enormous and this variability is reflected in cancer. Finally, this range of alterations is enhanced in disseminated disease, because patients have many metastatic locations that often limit our ability to obtain a tumor sample. We usually perform a biopsy from the most accessible metastasis. This limitation would force us to decide targeted therapy from genetic or genomic data from a single, easily approachable metastatic location in spite that the patient would have more than 5, 10, or 20 metastatic locations. So, should we collect a sample that reflects the dynamic condition of a global disease in a concrete patient? Please, do not forget the critical question: will this patient respond to this combination of drugs?

Proteomics for the Drugs: Help Us to Design Phase I Trials

Phase I trials are studies designed to test for the first time a new drug in humans (“first-in-human trials”) or to identify the exact dose, schedule, and/or route of administration for a new drug previously tested for the first time in humans. Most of these trials are designed on a Bonferroni modified model. Briefly, investigators administer 10% of the lethal dose for a mouse in a group of three patients. If toxicity is not observed, then the dose is increased for next patients until a preplanned final dose or unacceptable toxicity. The final dose is the Maximal Tolerated Dose and the toxicity that prevents larger doses is the dose-limiting toxicity. Additionally, almost all phase I trials include pharmacokinetic subanalysis to evaluate serum levels of the drug. In this regard, new designs for phase I trials are based on “adaptive” methods to speed up the process of getting a drug into the clinic, and a few investigators have evaluated the possibility to increase initial drug doses for the phase I trial to effectively get a target serum level. All these designs (classical, adaptive, pharmacokinetical, and so on) try to get a serum level for the experimental drug, limited by toxicity or by extrapolated, preplanned final dose from animal models. We are administering drugs for patients based on estimations from serum levels in absence of data of intratumoral effective levels. However, tumors show an increased interstitial fluid pressure, which forms a barrier to transcapillary transport because of vessel abnormalities, fibrosis, and contraction of the interstitial matrix. So, is the serum level we detect enough to insert a single molecule on cancer cells? Furthermore, does anyone know the exact level that a drug reaches on tumor cells? Can we avoid the possibility that the most relevant factor that induce resistance to chemotherapy is the risk that chemo does not reach the tumor in human patients? Furthermore, the way to calculate the dose for targeted therapies is the same (maximum tolerated dose and dose-limiting toxicity) that we have used for cisplatin, paclitaxel, and other drugs in spite the fact that they are mechanistically different. Imagine that you have a technology that may supply information to identify in rodent models the exact dose you need to administer for a drug to reach the tumor. May MS imaging help us to modify the actual design for phase I clinical trials? If MS imaging data suggest that the correct dose for a xenografted mouse to observe a molecule of the drug X within the tumor is Y, do we test 10% of the Y dose for a patient? Dare you do it? Please, do not forget the key issue: what is the right dose for this patient to respond?

Conclusions

Chemotherapy being administered to entire populations composed with similar-histology from similar primary locations cancer patients is now on decay. The future will be made up of small subsets of patients defined by a several biomarkers that predict response for a drug or for a combination of medicines. Obviously, dynamic properties of cancer will require a source of samples for surrogated biomarkers that update the biological information accordingly with clinical behavior. Although many studies have provided proteomic information from blood samples collected in clinical practice, there are no biomarkers in clinical practice from these approaches. Probably, complexity of proteomic technologies in concurrence with a reciprocal lack of awareness is responsible for this result. Many oncologists are confident on the unavoidable incorporation of high-throughput proteomics in daily practice. However, the only way we may accelerate this process is an international approach to decipher each tumor proteome for every clinical scenario. This International Consortium of hospitals, research centers, and governments would design a database similar to that designed to correlate HIV genotypes, phenotypes, and treatments. Can you imagine that all patients in participating countries would have blood samples prior to surgery and/or to standard adjuvant chemotherapy? Can you imagine that all patients would have blood samples every 3 months for five years? Can you imagine that you could retrospectively analyze a particular sample when a recurrence is diagnosed? Many think that it is the time to move from proof of concept to proof of biology in clinical oncology, and we and our patients need you, your know-how, and your technology.

If many groups worked together to decipher the entire genome with technologies designed more than 20 years ago, can we work together in the era of global communications and global access to technology and health systems to decipher the serum proteome of early colon cancer, early nonsmall cell lung cancer, early breast cancer, and so on? Can we confront data from proteome platforms to clinical and genomic data to improve the pathways of clinical decisions in our current patients? Do you need more than 5 years? Probably not and you know that.

References
