Review Article

Current Challenges in Cancer Treatment

Jon Zugazagoitia, MD, PhD[1,2]; Cristiano Guedes, MD[1]; Santiago Ponce, MD[1,2]; Irene Ferrer, PhD[2]; Sonia Molina-Pinelo, PhD[2]; and Luis Paz-Ares, MD, PhD[1,2]

[1]Medical Oncology Department, Hospital Universitario 12 de Octubre, Madrid, Spain; and [2]Instituto de Investigación I+12. Lung Cancer Clinical Research Unit CNIO, I+12, Madrid, Spain

ABSTRACT

Purpose: In this review, we highlight the current concepts and discuss some of the current challenges and future prospects in cancer therapy. We frequently use the example of lung cancer.

Methods: We conducted a nonsystematic PubMed search, selecting the most comprehensive and relevant research articles, clinical trials, translational papers, and review articles on precision oncology and immuno-oncology. Papers were prioritized and selected based on their originality and potential clinical applicability.

Findings: Two major revolutions have changed cancer treatment paradigms in the past few years: targeting actionable alterations in oncogene-driven cancers and immuno-oncology. Important challenges are still ongoing in both fields of cancer therapy. On the one hand, druggable genomic alterations are diverse and represent only small subsets of patients in certain tumor types, which limits testing their clinical impact in biomarker-driven clinical trials. Next-generation sequencing technologies are increasingly being implemented for molecular prescreening in clinical research, but issues regarding clinical interpretation of large genomic data make their wide clinical use difficult. Further, dealing with tumor heterogeneity and acquired resistance is probably the main limitation for the success of precision oncology. On the other hand, long-term survival benefits with immune checkpoint inhibitors (anti–programmed death cell protein-1/programmed death cell ligand-1 [PD-1/L1] and anti–cytotoxic T lymphocyte antigen-4 monoclonal antibodies) are restricted to a minority of patients, and no predictive markers are yet robustly validated that could help us recognize these subsets and optimize treatment delivery and selection. To achieve long-term survival benefits, drug combinations targeting several molecular alterations or cancer hallmarks might be needed. This will probably be one of the most challenging but promising precision cancer treatment strategies in the future.

Implications: Targeting single molecular abnormalities or cancer pathways has achieved good clinical responses that have modestly affected survival in some cancers. However, this approach to cancer treatment is still reductionist, and many challenges need to be met to improve treatment outcomes with our patients. (*Clin Ther*. 2016;38:1551–1566)

Key words: cancer therapy, checkpoint inhibitors, drug development, immunotherapy, lung cancer, next-generation sequencing, precision oncology, targeted therapy.

INTRODUCTION

Cancer is a major public health problem worldwide. Global demographic characteristics predict an

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increasing cancer incidence in the next decades, with >20 million new cancer cases annually expected by 2025. According to GLOBOCAN data, 14.1 million new cases and 8.2 million deaths from cancer were estimated in 2012.[1] Cancers of the female breast, colorectal, prostate, and lung are the most frequently diagnosed cancers in Europe.[2] Lung cancer remains the leading cause of cancer incidence and mortality worldwide.[1]

The increasing knowledge of molecular and tumor biology has notably changed cancer treatment paradigms during the past 15 years. Formerly, cancer was classified and treated solely according to organs of origin or simplistic histomorphologic features. In a seminal paper published by Schiller et al[3] in 2002, completely overlapping survival curves were found in advanced non–small-cell lung cancer (NSCLC) patients after use of 4 different platinum-based chemotherapy doublets with third-generation drugs. Even though the trial was limited to lung cancer, it found that cancer treatment based on a broad use of cytotoxic chemotherapies in unselected patients had reached its therapeutic plateau. In addition, it became clear that the development of molecularly targeted therapies and treatment selection based on particular molecular alterations was needed. Since then, 2 pillars have driven the subsequent evolution of cancer treatment: new technology acquisition for tumor molecular profiling and the discovery of predictive molecular targets. Together, these efforts have materialized the 2 recent revolutions in cancer treatment. First, genotype-directed precision oncology, that is, tailoring personalized therapies to subsets harboring specific genomic abnormalities across different tumor types. Second, targeting components of the tumor microenvironment, in particular the immune system and the antitumor immunity. In this review, we will succinctly describe the fundamental premises of these 2 anticancer strategies. We will also highlight some of the major challenges ahead in both fields of cancer treatment, frequently using the example of lung cancer.

METHODS

We did a nonsystematic review of current concepts in precision oncology. References for this review were identified through searches of PubMed using the terms *precision oncology* (8301 results; 313 clinical trials), *oncogene addiction* OR *targeted therapies* (102,601 results; 4883 clinical trials), *next-generation sequencing* OR *early drug development* (69,901 results; 2201 clinical trials), *immunotherapy* OR *immuno-oncology* (255,507 results; 14,081 clinical trials), *immune checkpoint inhibitors* OR *PD-1/L1 blockade* (769 results; 17 clinical trials), and *non–small-cell lung cancer*. Articles were selected mainly on the basis of their clinical applicability, and we prioritized for practice-changing clinical studies, some translational papers, and selected comprehensive reviews published in the last 5 years. Relevant articles were also identified through searches of the authors' files and when reviewing other papers and their respective bibliographies. Unpublished reports from scientific conferences were identified across meeting libraries and abstract books. Only articles published in English were included. All of the references cited in this article were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this review.

TARGETING ACTIONABLE ALTERATIONS IN ONCOGENE-DRIVEN CANCERS

The essential premise of genotype-based precision oncology is that tumor-specific molecular abnormalities can be targeted with accurate, effective, and potentially less-toxic therapies. Extensive preclinical work and primary discoveries of somatic, single-gene genomic abnormalities that could be pharmacologically targeted opened the first gateways for genomic precision oncology. More recently, comprehensive and integrative characterization of many cancers using high-throughput technologies under the auspices of national (eg, The Cancer Genome Atlas, funded by the National Cancer Institute and National Human Genome Research Institute in the United States) or international (eg, International Cancer Genome Consortium) efforts, has led both to a new era of genomic or molecular taxonomy of cancer and to the discovery of cancer genes and biomarkers for therapy.[4]

There are 3 crucial issues for successful clinical biomarker development: biologic plausibility (the identified genomic alteration is responsible for malignant transformation and tumor progression), analytical validity (it can be detected with robust, reliable, and clinically applicable genomic tests), and clinical validity (the prognostic or predictive utility of the biomarker has been validated in clinical trials and community-based clinical cohorts). At the same time, it must be emphasized that clinical biomarkers might have diagnostic, prognostic, predictive, or pharmacogenomic utilities.[5] Predictive biomarkers are the most

useful markers in daily practice, as they simultaneously enable both selection of subsets that will obtain the greatest benefits from a certain treatment and exclusion of those who will not benefit from therapy. Prognostic markers, however, are informative of patient outcomes irrespective of treatment, and are therefore less frequently used in the clinic for treatment decisions.

NSCLC is one example that illustrates the paradigms of genomics precision oncology. From the initial one size fits all described in the study by Schiller et al,[3] shortly after 3 research groups found that the presence of mutations in the tyrosine kinase domain of *EGFR* gene (*EGFR*-activating mutations in exons 18−21) conferred sensitivity to EGFR tyrosine kinase inhibitors (TKIs).[6,7,8] Subsequently, a landmark trial was initiated enrolling metastatic pulmonary adenocarcinoma patients enriched for the existence of *EGFR* mutations (Asiatic, never or light smokers) who were randomly assigned to receive first-line chemotherapy or an EGFR TKI (gefitinib). A significant treatment interaction ($P < 0.0001$) was found between *EGFR* mutation status and treatment arm, so that *EGFR* mutation−positive patients selectively benefited from gefitinib in terms of progression-free survival (PFS) (n = 261; hazard ratio [HR] = 0.48; 95% CI, 0.36−0.64; $P < 0.0001$) compared with *EGFR* mutation−negative patient (n = 176; HR = 2.85; 95% CI, 2.05−3.98; $P < 0.0001$), underscoring the predictive effect of *EGFR*-activating mutations for the benefit of EGFR TKIs.[9]

This predictive benefit in terms of response rates (RRs) and PFS has been confirmed in at least 6 molecularly selected (patients with *EGFR* mutation−positive tumors) randomized trials, including Caucasian populations.[10] *ALK* gene fusions have undergone similar preclinical and clinical validation and, together with *ROS-1* rearrangements, are both well-established predictors for the benefit of ALK-ROS-1 TKIs.[11,12] Other targetable oncogenes, whose clinical data come from smaller cohorts or early clinical trials, include *B-RAF* and *HER2* mutations, and *MET* amplifications, which, with an approximate 40% RR with tailored therapies, are formally recognized as predictive targets in NSCLC. Finally, there are other oncogenic drivers whose targeted inhibition has shown encouraging results in preclinical models and clinical responses in few case series. These targets are currently being more widely studied in clinical trials, including, among others, *MET* mutations (exon 14 skipping), *RET*, and *NTRK1, 2, 3* rearrangements, *FGFR1, 2, 3* amplifications or mutations, and *DDR2* mutations (the last 2 predominantly in squamous cell carcinomas)[13] (Table I). Importantly, using multiplexed genomic testing, druggable genomic targets (including *EGFR, ALK, ROS-1, BRAF, HER2, MET, RET, PIK3CA* genomic alterations) were identified in up to 64% of the samples within a large multicenter cohort of lung adenocarcinoma patients. Those patients receiving appropriate matched therapies achieved longer survival compared with those with driver alterations but not receiving targeted therapies or those without oncogenic aberrations susceptible of specific treatment.[14] Finally, integrative and comprehensive molecular characterizations of both adenocarcinomas and squamous cell carcinomas have already been published, and it is thought that a genomics-based classification of human lung tumors will become a reality in the near future.[15] Of course, oncogene-targeted therapies have been successful for treating other subsets of solid tumors apart from NSCLC[4,16] (Table I).

Three important issues must be pointed out regarding oncogene-targeted therapies. First, most of these examples illustrate the key concept of oncogene addiction. In these tumor models, a single driver mutation confers distinct biologic properties and is capable of driving the main oncogenic capabilities so tumor cells become strongly dependent on that specific genomic alteration for survival.[17] Second, these genomic drivers represent small subsets of patients across different solid tumors.[4,16] And third, the vast majority of genomic alterations that are clinically validated as predictive markers are genes encoding for molecules involved in pathways related to sustained proliferation or apoptosis inhibition[18] (Table I). One possible exception is the tumor carrying trunk mutations in genes involved in DNA repair mechanisms. For instance, germ-line *BRCA1, 2* mutations have been successfully validated as predictive markers for the benefit of poly ADP ribose polymerase inhibitors (which are synthetic lethal in the presence of homologous repair deficiency) in advanced, high-grade, serous ovarian carcinomas.[19,20]

CANCER IMMUNOTHERAPY

The longstanding hypothesis of cancer immunoediting is now recognized as a core process of tumorigenesis. It is well known that many solid tumors are

Table I. Oncogene addicted tumors and matched targeted therapies.

Tumor Type and Gene	Genomic Alteration	Prevalence, %	Selected Drugs with Available Clinical Data in Molecularly Selected Patients	Response Rate, %
NSCLC				
EGFR	Activating mutations	10–15	Gefitinib, erlotinib, afatinib, dacomitinib, NG EGFR TKI[†]	50–70[*]
ALK	Gene rearrangements	2–5	Crizotinib, NG ALK TKI[‡]	50–70[*]
ROS-1	Gene rearrangements	1–2[*]	Crizotinib, NG ROS1 TKI	50–70[*]
HER2	Activating mutations	1–2	Afatinib, dacomitinib, neratinib, trastuzumab[§]	20–40
BRAF	Activating mutations	1–2	Vemurafenib, dabrafenib[§]	40
MET	Amplification and point mutation	1–5[‖]	Crizotinib, cabozantinib, INC280[§]	30–40
RET	Gene rearrangements	1–3	Cabozantinib, vandetanib, sunitinib, sorafenib[§]	–
NTRK1, 2, 3	Gene rearrangements	1–3	Entrectinib, LOXO-101[§]	–
FGFR1, 2, 3[¶]	Amplification and activating mutations	5–20	BGJ398, AZD4547[§]	–
DDR2[¶]	Activating mutations	1–3	Dasatinib[§]	–
Melanoma				50–65
BRAF	Activating mutation *(V600)*	60	Vemurafenib, dabrafenib+trametinib[#]	
Breast cancer				
HR	Overexpression	60	Hormonal therapies[**]	50–70
HER2	Amplification	20	Trastuzumab, pertuzumab, lapatinib	80
Prostate cancer		70	Hormonal therapies[††]	
AR	Overexpression, Amplification		Abiraterone,[‡‡] enzalutamide[‡‡]	50–70[§§]
Gastric cancer				
HER2	Amplification	20	Trastuzumab	40–50
GIST				
c-kit and PDGFR	Activating mutations	80	Imatinib	50–85

AR = androgen receptor; GIST = gastrointestinal stromal tumor; HR = hormonal therapies; Inh = inhibitors; NG = next generation; TKI = tyrosine kinase inhibitors.
[*]Data correspond to Caucasian populations.
[‖]De novo. MET amplification might occur in up to 10% of EGFR-acquired resistance.
[¶]Predominantly mutated in squamous-cell lung carcinomas.
[†]Including osimertinib and rociletinib.
[‡]Including alectinib and ceritinib.
[§]Not approved drugs.
[#]Trametinib is a MEK inhibitor.
[**]Including LHRH agonists, tamoxifen, aromatase inhibitors.
[††]Including LHRH agonists, bicalutamide.
[‡‡]Castration-resistant prostate cancer.
[§§]Lower responses for castration-resistant prostate cancers.

immunogenic.[21] During malignant transformation, non-self, tumor-associated antigens or neoepitopes resulting from gene mutations are created, which can be recognized by the immune system. This process is called immune surveillance. At least initially, adaptive, tumor antigen–specific T-cell responses are generated, leading to cancer-cell elimination.[21,22,23] Several steps are recognized in this anti-tumor immune response that have been comprehensively depicted in a seminal review by Chen and Mellman,[24] commonly known as the cancer immunity cycle. However, it is clear that tumors finally escape from immune attack, despite functional immune systems. Incomplete tumor elimination is followed by an equilibrium phase, in which cancer cells shape their microenvironment and initiate complex mechanisms of immune evasion that will finally lead to immune escape and tumor progression.[21,25] Actually, immune evasion is considered one of the core hallmarks of cancer[18] and, importantly, targeting these immune-suppressive mechanisms is revolutionizing cancer treatment. Cancer cells can generate immune-suppressive networks in every step of the cancer immunity cycle. Thus, tumors can disrupt the generation of tumor-reactive T cells (defective dendritic cell maturation and activation, defective T-cell activation), T-cell trafficking (immune-suppressive chemokine milieu) or T-cell cross of the tumor vasculature (tumor endothelium is both a physical barrier for T cells and an active immune suppressor by generation of angiogenic (eg, vascular endothelial growth factor) and immunosuppressive factors (eg, prostaglandin E2, indoleamine 2,3-dioxygenase, TIM-3, and PD/L1-2). For those T cells that manage to "home" the tumor, they reach an immune-suppressive local tumor microenvironment dominated by tumor-associated immune-suppressive leukocytes (regulatory T cells, myeloid-derived suppressor cells), and a number of soluble immunosuppressive molecules (generated mainly by these cells, which include transforming growth factor–β, interleukin-10, adenosine, and indoleamine 2,3-dioxygenase, among many more), that prevent the encounter with their tumor-antigen target or suppress their immune functions. Finally, cancer cells can evade the final step of immune attack and immune rejection by avoiding antigen recognition (eg, downregulation of major histocompatibility complex class I molecules) or, for instance, by upregulating a number of membrane receptors that induce apoptotic signals in T cells (FasL and tumor necrosis factor–related apoptosis-inducing ligand) or immune tolerance (PD/L1–2).[24,25]

Immune-suppressive inhibitory checkpoint molecules generated upon T-cell activation that regulate the immunologic synapse between T cells and dendritic cells in lymph nodes (eg, cytotoxic T lymphocyte antigen-4 and B7.1), modulating T-cell activation; or between T cells and tumor cells in the tumor bed (eg, PD-1/L1–2), modulating immune rejection or the effector phase, are among the most relevant targets for immunotherapy.[26-28] Monoclonal antibodies blocking cytotoxic T lymphocyte antigen-4 and PD-1/L1 receptors have yielded clinical benefits in several tumor types and, contrary to classic cytotoxic chemotherapy or even targeted therapy, they provide durable, long-term survival benefits. Particularly appealing clinical results have been reported with PD-1/L1 blockers and treatment paradigms have changed in an increasing list of solid tumors, particularly melanoma, NSCLC, or kidney cancer[27-29] (**Table II**). The long-term overall survival (OS) benefit achieved with nivolumab compared with standard second-line chemotherapy in advanced NSCLC is an example of this encouraging clinical efficacy. In the CheckMate 017 trial, 272 advanced, previously treated, squamous-cell lung cancer patients were randomly assigned to receive nivolumab (n = 135) or docetaxel (n = 137). The patients who received nivolumab had a 3-month improvement in OS (HR = 0.59; 95% CI, 0.44–0.79; $P = 0.00025$), and 1-year survival rates were 42% compared with 24%. There was also a treatment benefit in terms of RR and PFS in favor of nivolumab.[30] With a similar study design, the CheckMate 057 trial confirmed the OS improvement in the non–squamous-cell lung cancer subsets (12.2 months vs 9.7 months, respectively; HR = 0.73; 95% CI, 0.59–0.89; $P = 0.002$). Intriguingly, median PFS did not favor nivolumab (2.3 months vs 4.3 months, respectively),[31] which underscores the paradigm shift of immunotherapy compared with other anticancer drugs relative to its long-term survival benefits.

Remarkably, PD-1/L1 blockers have a better toxicity profile than conventional chemotherapy.[29] While immune-related adverse events might be relatively frequent, they are mostly mild and can be well managed with adequate training. Apart from immune checkpoint inhibitors, many other important immune-based therapies are being developed, which can be practically classified into active or passive immunotherapies according to the implication of host's immunity when eliciting anti-tumor responses.

Table II. Practice changing Phase II–III randomized trials with anti–cytotoxic T lymphocyte antigen-4 and anti PD-1/L1 agents in solid tumors.

	Agent	Tumor Type	Treatment Scenario	Phase	Control Arm	Response Rate	Median OS	Study First Author
PD-1/L1	Nivolumab	Melanoma	First line	III	Dacarbazine	40% vs 13.9%	NRe vs 10.8 mo 73% vs 42% 1-y OS	Robert[64]
		NSCLC	Pretreated[*]	III	IC-CT	32% vs 11%	NR	Weber[63]
		Squamous	Pretreated	III	Docetaxel	20% vs 9%	9.2 mo vs 7.3 mo	Brahmer[30]
		Non-squamous	Pretreated	III	Docetaxel	19% vs 12%	12.2 mo vs 9.4 mo	Borghaei[31]
		RCC	Pretreated	III	Everolimus	25% vs 5%	25 mo vs 19.6 mo	Motzer[61]
	Pembrolizumab	Melanoma	Pretreated[*]	II	IC-CT	25% vs 4%	NR	Ribas[68]
			First line	III	Ipilimumab	33.7% vs 11.9%	NR in any group 74.1% vs 58.2% 1-y OS	Robert[69]
		NSCLC	Pretreated	III	Docetaxel	18% vs 9%	12.7 mo vs 8.5 mo	Herbst[70,†]
CTLA4	Ipilimumab	Melanoma	Pretreated	III	Gp100	10.9 mo vs 1.5 mo	10 mo vs 6.4 mo	Hodi[71]
			First line[‡]	III	Dacarbazine	15.2% vs 10.3%	11.2 mo vs 9.1 mo	Robert[72]
	Nivolumab+ Ipilimumab	Melanoma	First line	III	Ipilimumab	61% vs 11%	NR	Postow[73]
			First line	III	Monotherapy[§]	57.6% vs 43.7% vs 19 %	NR	Larkin[74]

IC-CT = investigator's choice chemotherapy; OS = overall survival; NSCLC = non–small-cell lung cancer; NR = not reported; NRe = not reached; RCC = renal-cell carcinoma.

[*]Progressed after ipilimumab, or ipilimumab and a BRAF inhibitor if they were BRAF (V 600) mutation-positive.

[†]Large international Phase I trial leading to pembrolizumab approval in NSCLC. Among PD-L1 + patients (≥50%), response rate = 45.2% and median OS not reached.

[‡]The experimental arm consisted in ipilimumab+dacarbazine.

[§]Nivolumab and ipilimumab, respectively.

Active immunotherapies (which include immune checkpoint modulators) depend on the host's capability of mounting a T-cell–based antitumor attack. Contrary, passive immunotherapies, such as adoptive T-cell therapy, are those strategies that contain intrinsic anti-tumor properties.[32] Immunostimulatory antibodies against activatory checkpoint receptors (eg, anti-CD137 or anti-CD40, among others) are promising targets for anticancer immunotherapy and are being tested in clinical trials.[33]

Cancer vaccines are by far the most studied active immunotherapies in the clinic across a wide variety of tumor types, with mostly discouraging results.[34,35] Polyvalent, cell-based cancer vaccines (eg, dendritic cell or tumor-cell vaccines) containing a wide range of tumor-associated antigens are promising, but are not without technical difficulties for clinical applicability. There are comprehensive reviews in the field, which we recommend for interested readers.[36] As far as passive immunotherapies are concerned, adoptive T-cell therapy is among the most exciting anticancer immunotherapy. Early experiences in acute lymphoblastic leukemia have shown encouraging clinical efficacy results that merit further study in larger trials and other solid tumors.[37,38]

CHALLENGES AHEAD IN PRECISION ONCOLOGY

Clinical Implementation of Next-generation Sequencing Technologies

As stated previously, predictive genomic abnormalities are uncommon, diverse in nature, and distributed across many tumor types. These premises, together with the increasing number of predictive mutations in each tumor type, force the requirement of comprehensive, multiplexed, and highly sensitive sequencing techniques for routine clinical care.[4,5]

Classic Sanger sequencing does not fulfill these criteria and is definitely not cost-effective, as it lacks enough sensitivity, cannot test multiple genomic mutations simultaneously, and is unable to detect genomic abnormalities other than point mutations or small insertions or deletions.[39] To meet these requirements, high-throughput next-generation sequencing (NGS) technologies have been developed.[5]

Two next-generation molecular diagnostics can be distinguished: customized gene panels and whole exome and genome or transcriptome panels. Gene panels are thought to detect multiple potentially relevant mutations across several cancer genes (n = 20–300) at once, with sufficient scalability for clinical requirements. NGS gene panels can be either polymerase chain reaction (PCR) amplicon-based or hybrid capture-based. Each assay has its own advantages and disadvantages for routine clinical application. Because PCR amplicon-based panels rely on PCR amplification of commonly mutated exons of candidate genes before massively parallel sequencing, they are subject to limitations inherent to PCR DNA amplification (eg, false-positive results due to polymerase-induced artifacts in formalin-fixed paraffin-embedded tissue samples or false-negative results in samples with low tumor DNA input). In addition, their predominant applications include the identification of point mutations and deletions, so they need to be commonly combined with other techniques, such us immunochemistry of fluorescence in situ hybridization in order to cover a wider spectrum of predictive genomic abnormalities (eg, copy number variations or gene rearrangements).[5,39]

On the contrary, hybrid capture-based panels do not require amplicon generation and can reliably detect copy number variations and gene rearrangements, but demand more complex computational infrastructure.[39] Several customized gene panels are commercially available for Clinical Laboratory Improvement Amendment–certified laboratories and are increasingly being used in many academic centers for routine genotyping and patient care. These early clinical experiences have found that customized gene panel genotyping is not only feasible and adaptable to the limitations and challenges inherent to clinical settings (frequent limited biopsy tissue available, low tumor cellularity, formalin-fixed paraffin-embedded tissue samples, or rapid turnaround time required for therapeutic decisions, among others),[40] but is also clinically useful for routine cancer care or patient prompt selection for biomarker-driven early clinical trials.[41,42] However, at least for the moment, these experiences have been limited to large academic centers and are not yet widely available for the entire oncology community.

Whole exome and genome or RNA sequencing platforms are rarely used for routine clinical genotyping and are mostly used for research purposes. Several issues limit their clinical applicability. The first issue is the management of massive amounts of genomic datasets that require complex bioinformatics for proper data analysis, which, at least for the moment, is not compatible with

the short timeframe needed for therapeutic decision making. The second issue is the difficulty clinically interpreting the high quantity of noninformative, unknown significance genomic data that they generate. The third issue is the economic cost.[39,40]

Whichever NGS platform is used, clinical interpretation of the genomic results is definitely one of the most important challenges for the wide clinical applicability of NGS technologies. We highlight 3 characteristics that affect the clinical relevance of a particular genomic abnormality: functional impact (biological relevance), clinical effects, and targetability. Mutations affecting hotspot regions of biologically relevant cancer genes are, in general, functionally important, clinically relevant, and mostly targetable ("driver mutations"), but this functionality might be inherent to particular tumor types. However, it might be difficult to determine whether nonhotspot, previously undescribed or unknown significant mutations in biologically relevant genes are true drivers that can match with effective drugs, or are instead functionally or clinically irrelevant "passenger mutations."[39,43] This situation might be frequent in carcinogen-induced tumors carrying high mutational loads, such as melanoma or lung cancer. In addition, clinical diagnostic NGS adds further complexity because matched germline DNA is usually unavailable and the risk of falsely considering a rare polymorphic variant as a potential candidate driver cannot be excluded.[39] Several publically available databases and levels of evidence have been proposed in order to determine the clinical significance and actionability of genetic abnormalities.[43] What is evident is that expert multidisciplinary teams, including pathologists, molecular biologists, geneticists, bioinformatics, and oncologists, are needed for adequate data analysis, interpretation, and therapeutic decision guiding in the clinic.

Conducting Biomarker-driven Clinical Trials

The low mutation frequency of some genomic abnormalities, together with their wide distribution across many tumor types, raise major challenges when testing the clinical impact of precision cancer medicine. Designs of genotype-enriched clinical trials can be classified first according to whether they are histology-based or not. "Basket trials" are histology-independent, as they include patients with different tumors harboring a common genomic abnormality to receive a matched therapy within the framework of a Phase I–II trial.[44] As an example, an important Phase II basket trial for *BRAF V600* mutant nonmelanoma cancers was published recently.[45] Notably, one of the key findings of this trial is that the oncogenic properties and subsequent clinical targetability of genomic drivers are dependent on specific tumor types. As such, in this trial, encouraging activity in NSCLC subsets receiving vemurafenib (RR = 43%) was found, but not in other tumor types, such as colorectal cancers, anaplastic thyroid cancers, cholangiocarcinomas, or ovarian cancers.[45] On the other hand, histology-dependent "umbrella trials" restrict the inclusion criteria to a particular tumor type, and eligible patients are genotyped for multiple mutations that match with different drugs. These trials frequently incorporate randomization strategies (either biomarker-positive patients only or all biomarker-positive and negative patients) to include a control arm where patients receive nontargeted therapy in order to assess the prognostic or predictability of the biomarker in question.[44] Examples include the I-SPY 2 (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis 2; Clinicaltrials.gov ID: NCT01042379) and SAFIR-01 (High Throughput Technologies to Drive Breast Cancer Patients to Specific Phase I–II Trials of Targeted Agents; ClinicalTrials.gov ID: NCT01414933) trials in breast cancer or Master Protocol (A Biomarker-Driven Master Protocol for Previously Treated Squamous Cell Lung Cancer [Lung-MAP]; ClinicalTrials.gov ID: NCT02154490), and ALCHEMIST (Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trial; ClinicalTrials.gov ID: NCT02194738) trials in lung cancer.[43] Another strategy, which can be applied to basket or umbrella trials, is to randomly assign genotype-selected patients to molecularly tailored therapies versus conventional therapies instead of to specific drugs.[44] The recently reported, histology-agnostic, French SHIVA (A Randomized Proof-of-Concept Phase II Trial Comparing Therapy Based on Tumor Molecular Profiling Versus Conventional Therapy in Patients With Refractory Cancer) study is one example of these trials.[46] Finally, another different strategy to investigate the clinical value of personalized therapy is the "N-of-1" trial design, which compares, within each patient, the clinical outcome of a mutation-tailored therapy against the most recent nontargeted regimen.[44] The international WINTHER trial (A Study to Select

Rational Therapeutics Based on the Analysis of Matched Tumor and Normal Biopsies in Subjects With Advanced Malignancies; ClinicalTrials.gov ID: NCT01856296) is genomically and transcriptomically characterizing different tumor types across 6 countries to compare PFS rates between targeted versus most recent untargeted therapy with a modified N-of-1 design.[47]

All of these trial designs mentioned are mostly thought to investigate "early signals" of activity of a matched targeted therapy. New adaptive statistical designs or end points (eg, disease control rate at 8 weeks or early tumor shrinkage) are being increasingly used in order to optimize this proof-of-concept process.[44] Importantly, multicenter collaborative networks are essential to facilitate molecular prescreening (comprehensive molecular profiling) and subsequent patient selection for these clinical trials.[4] Besides the challenge, there are 2 other main issues that may limit new molecularly guided drug development. The first is target prioritization for trial eligibility.[43] As stated previously, misclassification of "driver and passenger" mutations can result in false-negative results, with the consequent drug rejection or exclusion of potential candidates that could truly benefit from therapy. The second issue is tumor molecular heterogeneity. Current practice assumes that a one-site, single-tumor biopsy is representative of the whole tumor genomic burden of the patient, underestimating the existence of intratumor heterogeneity. While driver mutations usually dominate all metastatic sites and this heterogeneity especially affects subclonal, probably passenger mutations, increasing knowledge of tumor clonal evolution highlights the fact that subclonal populations might affect the growth of clonally dominant cells, and are also responsible for acquired oncogene resistance.[43] Therefore, next-generation clinical trials that take into account the premise of tumor heterogeneity are being developed.[48] Finally, when positive signals from the early clinical trials mentioned are apparent, they usually need confirmation in larger, preferably Phase III randomized trials in order to get the drug approved for clinical use. Conducting large, Phase III randomized studies in molecularly selected cohorts with mutations that hardly represent 1% to 5% of overall cases might take several years, or even be unfeasible in some rare tumor types. Accelerated drug approvals are sometimes granted by the regulatory authorities when there is a strong biomarker or drug matching that translates into strong clinical efficacy.[44] Actually, the need for randomized controlled trials in this setting is a matter of unresolved debate in the oncology community.

Tumor Heterogeneity and Resistance

During cancer development, tumors acquire somatic mutations in an evolutionary Darwininan model. Cells that acquire certain mutations gain survival advantage and dominate localized tumor areas by displacing those lacking these genomic alterations. This process is enhanced by consecutive clonal expansions. According to this model, all cells within a tumor would be biologically similar, and thus equally susceptible to acquiring mutations and spawning new subclones. However, several lines of evidence indicate that tumor initiation and progression could rely on a relatively minor population of self-renewing cancer stem cells. These cancer stem cells would be the ancestors of a much larger population of more differentiated cells with limited proliferative capacity. To further complicate this picture, the following cancer stem cell models for tumor initiation and progression have been proposed: the strictly hierarchical model, in which cancer stem cells are a biologically distinct population within the tumor and the only ones with self-renewing and tumorigenic potential; and the non-hierarchical model, where potentially every tumor cell (particularly the transit-amplifying or progenitor cells, ie, the daughters of cancer stem cells) have plasticity and the potential ability to de-differentiate and re-enter the stem cell state in response to intrinsic or microenvironmental factors.[49] Darwinian and cancer stem cell models are not mutually exclusive, as they still rely on linear clonal successions. However, they are still oversimplistic because, if true, homogeneous clones would dominate tumor masses. Instead, tumor progression is accelerated by the progressive genomic instability inherent in cancer cells or exogenous carcinogens (eg, sunburn and smoking), which increase the generation of new mutations, probably exceeding Darwinian selection.[18]

In turn, the course of tumor progression, rather than being linear, occurs as a branched model, with tumor masses composed of increasing numbers of genetically distinct subclones. Actually, several high-throughput whole-genome sequencing studies across different tumor types have reported this, revealing high genomic variability within primary tumors

and metastatic regions.[50] In a breakthrough article, integrative genomic characterization of multiple primary and metastatic lesions of 2 renal-cell carcinomas found that there was genomic, transcriptomic, and functional heterogeneity within separate tumor sites, resulting in different tumor subclones. In addition, 25% to 50% of the detected mutations were private (tumor regions not detected in order).[51]

This high level of intratumor heterogeneity has also been confirmed in other tumor types, including lung cancer. Multiregion whole-exome sequencing in 11 localized adenocarcinomas did reveal high levels of intratumor heterogeneity but, importantly, the majority of mutations (76%) were present in all tumors regions, and most of the driver mutations tended to be truncal, occurring early in tumorigenesis.[52] This was also found in the study by Gerlinger et al[51] and similar reports in other solid tumors that suggest that despite high levels of tumor heterogeneity, recurrent targets and driver mutations are dominant and present in every tumor clone and tumor region, representing robust therapeutic targets.[50] This means that a single-site biopsy would be sufficient to detect those driver mutations that can match with their respective targeted therapies or, in other words, there is no need to perform multiple biopsies and check for heterogeneity for therapeutic decisions in this setting. However, increasing evidence suggests that some subclonal populations may support the growth and survival of neighboring, clonally dominant cells, turning some regions more aggressive than others. In addition, subclonal populations and branched clonal evolution are partly responsible for drug resistance.[43]

Tumor heterogeneity, together the emergence of resistant clones on drug pressure (clonal evolution) are definitely the main challenges for the success of precision oncology, as they are both closely related and explain the therapeutic failures in clinical practice. In the case of solid tumors, lung cancer has served as the main source to study acquired resistance to TKIs in oncogene-addicted tumors. Actually, the genetic basis and some clinically relevant mechanisms of acquired resistance in EGFR- or ALK-mutant lung cancers have been characterized, enabling the development of selective drugs to overcome resistant clones.[53] This has been done either by studying clinical samples (re-biopsies taken from patients on TKIs at disease progression)[54] or in experimental models.[55] In general, mechanisms of acquired resistance can be divided in 3 groups: those that imply second genomic alterations in the drug target (eg, secondary mutations in the TKI domain of EGFR or ALK), those that reactivate the same pathway (eg, *MEK* mutation in BRAF-mutant melanoma treated with BRAF inhibitors) or those involving activation of bypass track signaling pathways.[53] Nongenomic alterations (transcriptional and epigenetic changes) and intratumoral immunity have been described as other sources of acquired resistance to targeted therapy, but these mechanisms are less well established.[56] Among the first group, *EGFR T790M* mutations (exon 20) are detected in up to 60% of patients with acquired resistance to EGFR TKIs.[57] Targeted therapy against this secondary genomic event with third-generation EGFR TKIs (osimertinib or rociletinib) has changed the natural history of resistant EGFR disease, with 60% RR and preliminary 10-month median PFS in T790M+ subsets.[58,59] As far as the second group is concerned, functional activation of RAS-RAF-MEK-ERK pathway represents probably the most important bypass track leading to acquired resistance, both in EGFR and ALK-mutant lung cancers.[57,60] Combination strategies targeting bypass tracks have shown encouraging results in experimental models,[55] and are currently being investigated in clinical trials. Importantly, using these combinations as upfront treatments may delay the emergence of acquired resistance or turn tumors less aggressive or heterogeneous upon disease progression.

With all of this in mind, it is evident that repeated biopsies at progression are needed to determine resistant mechanisms and their potential targeted inhibition. This can be a challenge in routine clinical practice, as it might result in increased morbidity or be unfeasible in certain cases.[16] Genomic analysis of circulating tumor cells or circulating-free DNA ("liquid biopsies") could potentially minimize these issues, and these platforms are being developed in ongoing studies in the resistance setting and next-generation biomarker-driven clinical trials.[43] Other advantages include the possibility of optimal genotyping in the absence of adequate tumor tissue and monitoring the molecular evolution while patients are on treatment.[61] Finally, in order to avoid or delay the emergence of more aggressive mechanisms of resistance, next-generation targeted therapies (third-generation TKI monotherapies and potentially combinations) are being investigated as first-line treatments

rather than therapies upon disease progression in EGFR- and ALK-mutant lung cancers. Liquid biopsies could be helpful to characterize the level of tumor heterogeneity in these patients, thus enabling the selection of potentially personalized upfront therapies in combination. It remains to be seen whether these blood-based genomic profiling or other approaches to face heterogeneity and monitor resistance will be scalable for clinical purposes. Table III details the most important challenges of genomics precision cancer medicine.

Predictive Markers for Immunotherapy and Precision Immuno-oncology

PD-1/L1 blockade has represented a real breakthrough in cancer treatment. However, long-term survival benefits are achieved only in a small fraction of patients (10%−20%). Deciphering what characterizes these subsets and developing molecular biomarkers that can help us identify which patients will obtain larger treatment benefits and thus optimize treatment selection is probably the main challenge in the field of cancer immunotherapy currently. Expression of PD-L1 assessed by immunochemistry has been the most studied marker in this regard, and its potential predictive capacity has been analyzed in most of the randomized controlled trials investigating the role of PD-1/L1 blockers. However, the predictive role of PD-L1 expression is far from being clarified at the moment.

Evidence across different tumor types suggests that although the higher tumor PD-L1 expression the better RR and survival rate, treatment benefits are not necessarily restricted to PD-L1−positive subsets.[62] In metastatic melanoma, 2 nivolumab pivotal trials reported that approximately 20% to 30% of PD-L1−negative patients responded, as compared with 50% of PD-L1−positive subsets.[63,64] Importantly, improved OS was reported with nivolumab compared with dacarbazine for PD-L1−negative patients in the CheckMate 066 trial (1-year OS

Table III. Premises and challenges for genomics precision oncology.

Premise	Clinical requirements	Main Challenges Ahead
Diverse and increasing number of predictive genomic abnormalities across tumor types	Implementing next-generation sequencing technologies for routine care	Technical issues Managing large amounts of genomic datasets Clinical interpretation of the genomic data Economic costs and accessibility
Low prevalence of some genomic abnormalities and widely distributed across many tumor types	Biomarker-driven trial designs to test their clinical impact	Large, multi-institutional efforts required Long recruitment period; time and costs Optimizing molecular prescreening methods and timing for patient selection Target prioritization
Tumor heterogeneity	Liquid biopsies and next-generation clinical trials	Tumor heterogeneity Robust assay development Clinical translation for routine care
Emergence of acquired resistance	Next-generation drugs targeting resistant clones and combinatorial strategies (upfront or at disease progression)	Emerging toxicities and tolerability

67.8% vs 37.4%, respectively).[64] In patients with squamous-cell NSCLC and renal-cell carcinoma treated with nivolumab, RRs and OS improved irrespective of PD-L1 expression.[30,65] However, in non-squamous NSCLC, a strong predictive effect for PD-L1 expression was suggested. For the PD-L1−positive subgroup, OS was doubled in favor of nivolumab (17.2 months compared with 9 months with docetaxel; HR = 0.59; 95% CI, 0.43−0.82). However, no differences were observed between nivolumab and docetaxel in PD-L1−negative tumors (10.4 months vs 10.1 months, respectively; HR = 0.90; 95 CI, 0.66−1.24; *P* value for interaction <0.001).[31] In line with these data, RRs and OS outcomes were notably higher for NSCLCs with ≥50% PD-L1 expression treated with pembrolizumab in the KEYNOTE 001 and 010 trials.[66,70] Several issues limit the interpretability of these results. The first ones are assay-related, as different antibodies or cutoff values have been used across clinical trials.[62] Second, RR might not be the ideal clinical end point to measure the predictive capacity of anti−PD-1/L1 drugs. And third, both PD-1/PD-L1 are upregulated upon interferon (IFN)-γ release and are therefore inducible rather that tight molecular markers.[28,62]

In this sense, PD-L1 might be overexpressed in 2 different tumor contextures. First, aberrant oncogenic pathways may intrinsically lead to PD-L1 upregulation,

Table IV. Potential predictive markers for PD-1/L1 blockade.

Biomarkers	Premise	Study First Author
PD-L1 expression	High PD-L1 expression might be correlated with better response rate and longer survival that absence of PD-L1 expression. However, some PD-L1−negative patients might still have durable responses and survival benefits. Assay-related inconsistencies and the fact that PD-L1 is an inducible marker that may vary according to tumor contextures limit the interpretability of the results across clinical trials.	Fusi[62]
Genomic complexity	High mutational burden has been shown to correlate with longer overall survival as compared with low mutational burden. This has been shown in carcinogen-induced tumors (melanoma, NSCLC) and in tumors developed in a context of DNA repair deficiency (colorectal carcinoma with microsatellite instability). Of note, mismatched deficient colorectal carcinomas show higher tumor-infiltrating lymphocytes. Importantly, in the case of NSCLC, smokers tend to respond better that nonsmokers, which is a paradigm shift as compared with oncogene-addicted lung adenocarcinomas, which are almost exclusive to nonsmokers.	Rizvi[75] Le[76] Snyder[77*]
Transcriptomic signatures	Type I interferon-based transcriptomic signatures might be associated with treatment benefits. This has been recently shown in metastatic melanoma but might be in principle applicable to other tumor types.	Ribas[78]
Tumor infiltrating lymphocytes	Pre-existing $CD8^+$ T cells distinctly located at the invasive tumor margin are associated with tumor regression upon PD-1/L1 blockade in metastatic melanoma. Importantly, using next-generation sequencing for T-cell antigen receptors, infiltrating T cells were found to be clonal, suggesting a tumor-antigen−specific T-cell responses, which, in turn, were inhibited by adaptive PD-L1 expression by tumor cells	Tumeh[79]

NSCLC = non−small-cell lung cancer.
*Data for anti-CTLA4 blockade.

resulting in constitutive PD-L1 expression. And second, PD-L1 might be upregulated upon IFN-γ release as a mechanism of adaptive tumor resistance against pre-existing anti-tumor immunity, reflecting immune rejection.[28,67] One could argue that adaptive, rather than constitutive, PD-L1 expression might be a surrogate marker for better outcomes with PD-1/L1 blockers. However, there is no clear explanation as to why some minority of PD-L1−negative patients still obtain treatment responses. The complex interaction networks of the immune system could explain that, even in the absence of PD-L1 expression, some antitumor immunity could be reinstated upon PD-1/L1 blockade by the activation of other pathways that could lead to tumor immune rejection.[62] Therefore, PD-L1 expression assessed by immunochemistry does not seem to satisfy all of the methodologic and biologic properties required to become the ideal biomarker. It is likely that establishing common testing criteria and better defining which cells (tumor cells or immune infiltrates), localization (tumor bed or adjacent to immune infiltrates in the invasive margins), or in what contexture PD-L1 is overexpressed would improve its utility as a predictive marker. Beyond PD-L1 expression, other promising biomarkers, including the pre-existence of tumor-infiltrating lymphocytes, high tumor mutational loads, or type I IFN-based transcriptomic signatures, have been reported to be potential positive predictive markers for the benefit of these drugs (Table IV).[28] Essentially, all of them are surrogates of a common denominator, namely, immunogenicity (mutational burden) or anti-tumor immunity (tumor-infiltrating lymphocytes, IFN signature) in a context of adaptive immune resistance (Figure 1). In this sense, probably one of the most promising biomarkers for immunotherapy is measuring the genomic contexture of the tumor. Because somatic mutations have the potential to encode immunogenic neoantigens, tumor genomic profiling might determine the sensitivity not only to immune checkpoint inhibitors, but also for other promising immunotherapies. Actually,

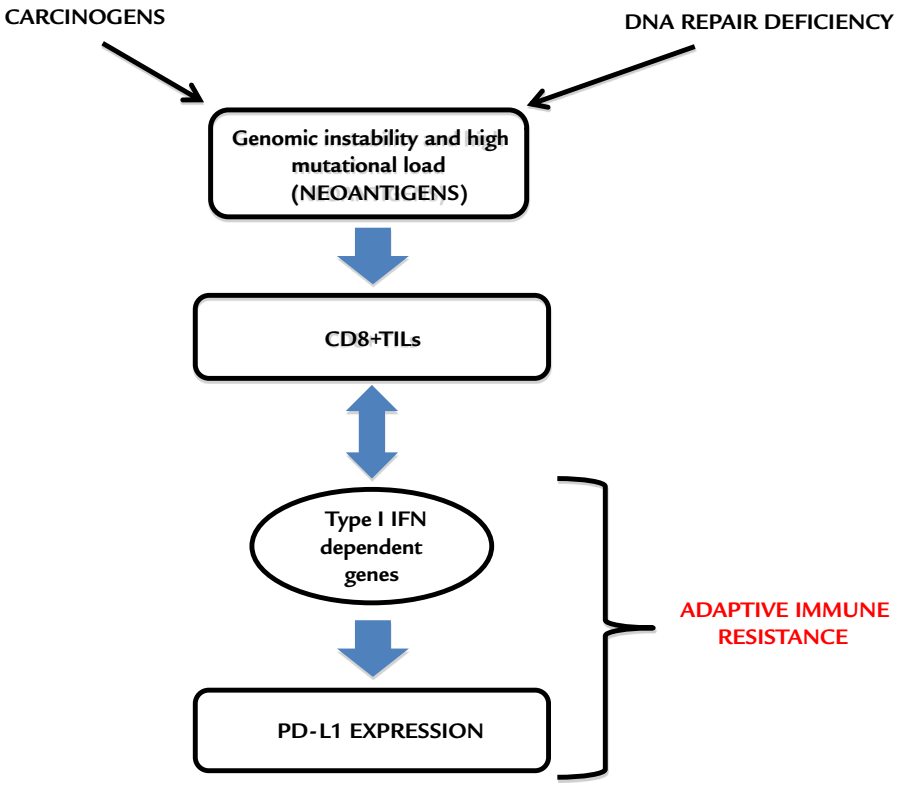

Figure 1. Surrogates of pre-existing immune activation and adaptive immune resistance as markers for the benefit of immune chelpoint inhibitors.

engineered, tumor-antigen–specific adoptive T-cell therapies (T cells that express cloned T-cell receptors or chimeric antigen receptors for specific tumor antigens or epitopes) based on the specific patient's mutanome, is probably one of the most promising strategy for precision immunoncology.[37,38]

CONCLUSIONS

Despite major advances, the current approach to face cancer treatment is still reductionist. Targeting single molecular abnormalities or cancer pathways has achieved good clinical responses that have modestly affected survival in some cancers. However, targeting a single hallmark or pathway with a single drug ("magic bullet") will not likely lead to cancer cure. We predict that drug combinations against several molecular alterations or cancer hallmarks, in a way that is similar to what we have done with HIV treatment, might be a promising therapeutic strategy to treat cancer in the near future. Given the encouraging success of immunotherapy (particularly checkpoint inhibitors), PD-1/L1 blockade may constitute a basic pillar for these combinations. Immunotherapies could be combined either among each other (2 checkpoint inhibitors or a checkpoint inhibitor plus an immunostimulatory antibody) or with other anticancer agents (including targeted agents in oncogene-driven cancers). Toxicity will be one of the key limiting factors when implementing these combination strategies in the clinic, and early recognition and management of adverse events will surely be a core component for treatment success. Ultimately, we evoke that personalized combination strategies according to pathways or hallmarks that specifically drive each patients' tumor biology within next-generation precision oncology initiatives will be one of the main challenges and most promising strategies for cancer treatment in the future.

CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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Address correspondence to: Luis Paz-Ares, MD, PhD, Medical Oncology Department, Hospital Universitario 12 de Octubre, Avenida de Córdoba, s/n, 28041, Madrid, Spain. E-mail: lpazaresr@seom.org