"Chance favors only the prepared mind"
Pasteur
Patient-centered approaches in oncology: new horizons

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Servier research and innovation: meeting unsatisfied needs in anticancer therapy

by E. Canet and P. Touchon, France

Servier is a French pharmaceutical company dedicated to therapeutic progress for the benefit of patients. Controlled by a nonprofit foundation, we are able to engage in therapeutic innovation activities in the long term and allocate at least 25% of our turnover to R&D every year. Our company was founded by Doctor Jacques Servier in 1954 with an initial focus on cardiovascular diseases and diabetes. Today, cardiology and diabetes are still our core areas of activity, but other major areas have been added, such as neurology, rheumatology, and cancer.

Servier and oncology: a long-standing commitment
In 1988, Muphoran® (lotemustine) was launched for the treatment of malignant melanoma and brain tumors. This drug continues to be provided today to patients in more than 30 countries in Europe and in emerging markets.

Since then, cancer research at Servier has been unrelentingly pursued. Under the guidance of Doctor Servier and now of Mr Olivier Laureau, we have been dedicating ever-increasing resources to oncology research to maximize efficiency in our fight against this disease. Our efforts have been rewarded by having found 8 new molecular entities in clinical development, including 5 “first-in-class” agents in various oncology indications.

Although many efforts in the past were made to identify new potent cytotoxic agents, the increasing understanding of carcinogenesis mechanisms led us to consider different and more specific approaches to targeting cancer.

The hallmarks of cancer
Cancer was long known to be a multifactorial disease; it was therefore clear that it would not be defeated through a single type of weapon, and that the remarkable diversity of neoplastic diseases would require to be addressed by several targeted therapies with complementary mechanisms of action. These concepts, increasingly supported by facts, were summarized by Hanahan and Weinberg in a landmark paper published in 2000 entitled “The hallmarks of cancer” (in Cell. 2000;100:57-70). This paper provided a logical framework for understanding and rationalizing the complexity of neoplastic diseases. The notions that “as normal cells evolve progressively to a neoplastic state, they acquire a succession of hallmark capabilities”—to wit: sustaining proliferative signaling; evading growth suppressors; resisting cell death; enabling replicative immortality; inducing angiogenesis; and activating invasion and metastasis. The authors further stated that “tumors are more than in-sular masses of proliferating cancer cells,” but are “complex tissues composed of...
multiple distinct cell types” contributed in a definitive fashion not only to the better knowledge of human tumor pathogenesis, but also exerted a strong influence on the development of new methods to treat cancer.

In 2011, in a follow-up paper entitled “Hallmarks of cancer: the next generation” (Cell. 2011;144:646-674), the same authors added two key concepts to the initially described six biological capabilities acquired during the multistep development of human tumors: “reprogramming of energy metabolism” and “evading immune destruction.” They further depicted how “in addition to cancer cells, tumors exhibit another dimension of complexity” as “they contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the tumor microenvironment.”

It then became clear that an attempt to impair tumor growth and progression by targeting only a particularly important biological capability, would in fact result in transitory clinical responses followed by almost inevitable relapses. Hanahan and Weinberg concluded that drug development “will benefit from incorporating the concepts of functionally discrete hallmark capabilities and of the multiple biochemical pathways involved in supporting each of them” and that “selective cotargeting of multiple core and emerging hallmark capabilities and enabling characteristics in mechanism-guided combinations will result in more effective and durable therapies for human cancer.”

Taking this into account Servier developed a diversified pipeline of products targeting multiple and complementary cancer hallmarks.

To maximize our discovery program’s efficiency, scientific collaborations have been set up with a great number of prestigious research institutes working on similar or complementary approaches. In addition, to extend the scope of explored targets and/or boost clinical development, several partnerships were established with biotechnology and pharmaceutical companies.

Servier decided not only to continue and reinforce its internal research efforts, with a primary focus on apoptosis, but also to develop other innovative and promising approaches involving tumor-based mechanisms (eg, targeted kinase inhibition), and tumor environment (eg, immune-modulation).

**Apoptosis as a barrier to cancer**

The concept according to which programmed cell death by apoptosis serves as a natural barrier to cancer development has been well established over the last two decades and elucidation of the mechanisms governing the cellular apoptotic program has revealed how apoptosis is triggered in response to various physiologic stresses that cancer cells experience during the course of tumorigenesis or as a result of anticancer therapy. Tumor cells evolve a variety of strategies to limit or circumvent apoptosis. Most common is the loss of TP53 tumor suppressor function, but tumors may also achieve similar ends by increasing expression of antiapoptotic regulators (Bcl-2, Bcl-xL, Mcl-1) or of survival signals (Igf1/2), by downregulating pro-apoptotic factors (Bax, Bim, Puma), or by short-circuiting the extrinsic ligand-induced death pathway.

With Vemalics (Cambridge, UK) as a partner, highly selective agents for direct therapeutic modulation of the BH3-mimetic family regulated apoptotic pathway for treatment of cancer have been discovered and are currently being developed. Around this program, collaborative activities with the Walter and Elisa Hall institute of Medical Research in Melbourne, one of the worldwide leading research centers in this field, and a partnership for clinical development of these drugs with Novartis, have been initiated.

**Turning off the angiogenic switch**

During tumor progression, an “angiogenic switch” is almost always activated and remains on, causing normally quiescent vasculature to continually sprout new vessels that help sustain expanding neoplastic growth as demonstrated by Hanahan and Folkman in 1996. Moreover, a compelling body of evidence indicates that the “angiogenic switch” is governed by countervailing factors that either induce or oppose angiogenesis. The well-known prototype of angiogenesis inducers is the vascular endothelial growth factor-A (VEGF-A) but other proangiogenic signals, such as members of the fibroblast growth factor (FGF) family, have been implicated in sustaining tumor angiogenesis.

In 2012, Servier in-licensed lucitanib (also named S 80881, CO-3810, and E-3810), from the Italian biotech Ethical Oncology Science (now acquired by Clovis Oncology Boulder, Colorado, USA). Lucitanib is a potent oral inhibitor of the tyrosine kinase activity of fibroblast growth factor receptors, types 1 and 2 (FGFR1-2), vascular endothelial growth factor receptors, types 1, 2, and 3 (VEGFR1-3) and platelet-derived growth factor receptors, alpha and beta (PDGFRα/β). The aim is, through the targeting of different parallel signaling pathways, to increase efficacy and to prevent the development of adaptive resistance. The preliminary clinical activity observed in the first-in-human clinical study for the treatment of breast cancer with this innovative drug, currently in phase 2, is very encouraging.

**Neoplastic tumors and immune system cells**

The presence of tumor-antagonizing CTLs and NK cells in neoplastic lesions is not surprising but, since the late 1990s, evidence has been accumulating that the infiltration of neoplastic tissues by cells of the immune system plays a key role in tumor inhibition and progression. Better understanding of T-cell activation and regulation has triggered the development of a new class of cancer immunotherapy agents.
T-cell activation requires costimulatory signals mediated by the binding of CD28 on the T-cell surface to B7 proteins (such as CD80 or CD86) on antigen-presenting cells (APCs). These two signals allow T cells to begin to proliferate, acquire antitumor effector functions, and eventually migrate to disease sites for tumor cell killing.

But T-cell activation is also tightly regulated by inhibitory signals in order to avoid prolonged immune responses that can potentially damage normal tissues. These inhibitory mechanisms can be mediated via cytokines, such as interleukin 10 (IL-10) or immunosuppressive cells, such as regulatory T cells and myeloid-derived suppressor cells, or via immune checkpoint molecules, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or programmed cell death 1 (PD-1).

Servier is addressing this field with two different, but complementary approaches. The first one through a collaborative agreement with Cellectis, a French biotechnology company, is focusing on the use of allogeneic engineered T cells armed with chimeric antigen receptors (CARs). CARs are genetically engineered T cells, expressing artificial T-cell receptors created by connecting the binding regions from a monoclonal antibody (mAb) through hinge and transmembrane domains to a cytoplasmic tail made up of the intracellular domains of T-cell receptor signaling molecules. T cells expressing a CAR will recognize and kill cells expressing the target antigen recognized by the mAb from which their binding regions were derived. CAR-based adoptive immunotherapy has already shown promising clinical results in particular for B cell malignancies (Porter et al, N Engl J Med. 2011;365 [8]:725-733).

The second approach is developed in collaboration with Macrogenics, a US-based company, using their Dual-Affinity Re-Targeting (DART) platform to combine targeting of an immune cell with that of a tumor cell to redirect T-cell activation and killing with a single molecule.

These few examples illustrate how, at Servier, we are trying to address unsatisfied needs in anticancer therapy. Such efforts led to an attractive/innovative portfolio combining small molecules, engineered mAbs, and cell therapy. More than 10 of them are already in the clinic and in the discovery pipeline between preliminary and preclinical development phases.

Moreover, in order to optimize the chances of success, a strong translational research effort has been set up with the aim of closely linking clinical development and strategic decisions on science-based evidence.

Conclusion
Working on a complex and multifactorial disease like cancer is no easy challenge. Today, through Servier’s strong commitment, hopes to develop, in coming years, innovative anticancer therapeutics that will have a significant and positive impact on the care of cancer patients, and on their quality of life and that of their families.

Keywords: angiogenesis; apoptosis; drug discovery; hallmarks of cancer; immune system cell; oncology; partnership
Servier a développé un portefeuille de produits innovants ciblant les processus biologiques clés à l’origine des cancers. Afin de relever le défi de l’innovation thérapeutique dans le cancer, Servier renforce ses efforts de recherche sur l’apoptose et l’immunothérapie des cancers, et développe, sur des cibles originales, des partenariats de recherche avec des institutions académiques, des sociétés de biotechnologie et des laboratoires pharmaceutiques.

Servier et l’oncologie : un engagement de longue date
En 1988, Muphoran® (fotémustine) a été mis sur le marché pour traiter le mélanome malin et les tumeurs cérébrales, et continue à être prescrit aux patients dans plus de 30 pays en Europe et sur les marchés émergents.

Depuis lors, sous l’impulsion du Docteur Servier et aujourd’hui de M. Olivier Lau reau, les ressources allouées à la recherche sur le cancer n’ont cessé de croître, dans le but de découvrir des médicaments innovants afin de lutter efficacement contre cette maladie. Ces efforts sont aujourd’hui récompensés par plus d’une dizaine de nouvelles entités moléculaires en développement préclinique et clinique, dont plus de la moitié sont des molécules « first in class », ciblant différents types de cancer.

Au-delà des nombreux efforts accomplis dans le passé pour identifier de nouveaux agents cytotoxiques puissants, la compréhension plus détaillée des mécanismes de la cancérogenèse amène à envisager des approches originales et plus spécifiques pour cibler les processus biologiques fondamentaux de la cancérogenèse.

Les « caractéristiques fondamentales » du cancer
Considérant que le cancer est une maladie multifactorielle, il est apparu très tôt qu’il ne pourrait pas être vaincu en utilisant une arme unique, et qu’il ne pourrait être mis en échec que par l’association de thérapies ciblées sur différents processus biologiques. Ces idées, de plus en plus corroborées par les faits, ont été synthétisées par Hanahan et Weinberg dans un article majeur publié en 2000 dans la revue Cell intitulé « The hallmarks of cancer » (Caractéristiques fondamentales du cancer) (Cell. 2000;100:57-70), qui a fourni un cadre permettant de mieux appréhender la complexité des maladies néoplasiques. Les notions telles que : « lorsque les cellules normales évoluent progressivement vers un état néoplasique, elles acquièrent succes-
sivement un certain nombre de ces caractéristiques » [à savoir : autosuffisance en signaux de croissance ; acquisition de la propriété de se répliquer indéfiniment ; résistance aux signaux inhibiteurs de croissance ; capacité à échapper à l’apoptose ; induction de la néo-angiogenèse tumorale ; pouvoir de formation des métastases], « les tumeurs sont davantage que des masses isolées de cellules cancéreuses proliférantes » [mais plutôt] « des tissus complexes composés de multiples types cellulaires distincts », ont assurément contribué non seulement à une meilleure connaissance de la pathogénèse tumorale chez l’homme, mais ont également profondément influencé le développement de nouvelles approches dans le traitement du cancer.


Ainsi on comprend mieux que cibler une seule de ces caractéristiques biologiques, même si importante, dans le but de modifier la croissance et la progression de la tumeur, conduit à des responses cliniques souvent transitoires. Hanahan et Weinberg concluent que le développement de médicaments contre le cancer ne pourra que « bénéficier de la prise en compte des caractéristiques fondamentales fonctionnelle-ment discrètes et des voies biochimiques multiples qui les sous-tendent » et que « un ciblage sélectif simultané de ces multiples caractéristiques devraient permettre la mise au point de thérapies plus efficaces du cancer chez l’homme ».

Compte tenu de la complexité de ces processus et pour accroître l’efficacité de notre programme d’innovation, des collaborations scientifiques ont été développées avec un certain nombre des instituts de recherche les plus prestigieux. De plus, pour étendre le champ des cibles explorées et/ou enrichir le portfolio de candidats médicamenteux en développement clinique, plusieurs partenariats ont été conclus avec des sociétés de biotechnologie et des laboratoires pharmaceutiques. Ainsi, le portefeuille de produits Servier cible plusieurs des processus biologiques fondamentaux de la cancérogenèse. Outre poursuivre et renforcer nos efforts de recherche sur l’apoptose, il a été décidé d’emprunter d’autres voies de recherche innovantes et prometteuses portant notamment sur l’inhibition ciblée des kinases et le micro-environnement tumoral, notamment la réponse immunitaire.

L’apoptose, barrière naturelle contre le cancer

Le concept selon lequel la mort cellulaire programmée (apoptose) constitue une barrière naturelle au développement du cancer a été bien circonscrit au cours des deux dernières décennies. Cette compréhension a permis de mieux appréhender les processus biologiques mis en jeu par les cellules tumorales en réponse à différents stress physiologiques qu’elles subissent au cours de la tumorigénèse ou à la suite d’un traitement contre le cancer.

Les cellules tumorales développent un certain nombre de stratégies destinées à échapper à l’apoptose. La plus commune est notamment la perte de la fonction du gène suppresseur de tumeur p53. Les tumeurs peuvent également résister au processus de mort cellulaire en augmentant l’expression des protéines anti-apoptotiques (Bcl-2, Bcl-xL, Mcl-1) ou des signaux de survie (IGF1/2), en effectuant une régulation négative des facteurs pro-apoptotiques (Bax, Bim, Puma), ou en court-circuitant la voie létale induite par des ligands extrinsèques.

Dans le cadre d’un partenariat avec Vernalis (Cambridge, UK), des agents hautement sélectifs permettant une modulation thérapeutique directe de la voie apoptotique régulée par la famille des protéines Bcl-2 ont été découverts et sont actuellement en développement clinique.

C’est dans ce contexte qu’une collaboration de recherche avec l’Institut de Recherche Médicale Walter et Elisa Hall de Melbourne, l’un des principaux centres mondiaux de recherche dans ce domaine, ainsi qu’un partenariat de développement clinique de ces candidats médicamenteux avec Novartis, ont été mis en œuvre.

Mise à l’arrêt de la commutation « angiogénique »

Au cours de la progression tumorale, une commutation angiogénique (« angiogenic switch ») se déclenche presque toujours, puis persiste, entraînant la formation continue de nouveaux vaisseaux par un système vasculaire normalement quiescent, ce qui contribue à favoriser la croissance néoplasique, comme l’ont démontré Hanahan et Folkman en 1996. En outre, un certain nombre d’arguments indiquent que cette commutation angiogénique est gouvernée par des facteurs qui induisent ou s’opposent à l’angiogénèse. Le prototype bien connu d’inducteur de l’angiogénèse est le facteur de croissance endothélial vasculaire A (vascular endothelial growth factor-A [VEGF-A]), mais d’autres signaux pro-angiogéniques, notamment les membres de la famille du facteur de croissance des fibroblastes (fibroblast growth factor [FGF]), participent au maintien de l’angiogénèse tumorale.

En 2012, Servier a acquis les droits auprès de la société de biotechnologie italienne Ethical Oncology Science (aujourd’hui acquise par Clovis Oncology, Boulder, Colorado, USA) du lucitanib (également dénommé S 80881, CO-3810 et E-3810), un inhibiteur puissant de l’activité tyrosine kinase des récepteurs du facteur de croissance des fibroblastes, de type 1 et 2 (FGFR1-2), des récepteurs du facteur de croissance endothélial vasculaire, de type 1, 2 et 3 (VEGFR1–3) et des récepteurs du facteur de croissance dérivé des plaquettes, al-
pha et bêta (PDGFRα/b). L’objectif a été, en ciblant différentes voies parallèles de signalisation, d’augmenter l’efficacité et de prévenir le développement d’une résistance adaptative. L’activité clinique préliminaire observée au cours de la première étude clinique menée dans le traitement du cancer du sein par ce médicament innovant, actuellement en phase II, est très encourageante.

**Lésions néoplasiques et cellules du système immunitaire**

La présence de lymphocytes T cytotoxiques et de lymphocytes « natural killer », des cellules combattant les tumeurs, dans les lésions néoplasiques n’est pas surprenante mais, depuis la fin des années 1990, des arguments de plus en plus nombreux montrent que l’infiltration des tissus néoplasiques par des cellules du système immunitaire joue un rôle essentiel dans l’inhibition de la progression tumorale. La meilleure compréhension de l’activation et de la régulation des lymphocytes T ouvre la voie au développement d’une nouvelle classe d’agents d’immunothérapie anticancéreuse dont pour certains l’efficacité a été démontrée en clinique.

L’activation des lymphocytes T nécessite des signaux de co-stimulation assurés par la liaison entre CD28 à la surface des lymphocytes T et les protéines B7 (notamment CD80 ou CD86) sur les cellules présentant l’antigène (CPA). Les deux signaux permettent aux lymphocytes T de commencer à proliférer, d’acquérir des fonctions effectrices antitumorales et finalement de migrer vers les sites pathologiques pour détruire les cellules tumorales.

Toutefois, l’activation des lymphocytes T est également strictement régulée par des signaux inhibiteurs afin d’éviter des réponses immunitaires prolongées, qui pourraient endommager les tissus normaux. Ces mécanismes inhibiteurs peuvent être médullés par le biais de cytokines, notamment l’interleukine 10 (IL-10), par des cellules immunosuppressives, notamment les lymphocytes T régulateurs et les cellules suppressives dérivées de la lignée myéloïde, ou par l’intermédiaire de molécules du contrôle immunitaire « immune checkpoint », notamment la protéine 4 associée aux lymphocytes T cytotoxiques (cytotoxic T lymphocyte–associated protein 4, CTLA-4) ou la protéine 1 de mort cellulaire programmée (programmed cell death 1, PD-1).

Servier aborde ce domaine avec deux approches différentes mais complémentaires. La première, par un accord de collaboration avec Cellectis, société française de biotechnologie, qui porte sur l’utilisation de cellules allogéniques T modifiées et armées par des récepteurs antigéniques chimériques (chimeric antigen receptors, CAR). Les CAR sont des récepteurs chimériques des lymphocytes T, créés en connectant les régions de liaison d’un anticorps monoclonal (ACm) spécifique d’un antigène tumoral aux domaines transmembranaires et intracellulaires des molécules de signalisation du récepteur des lymphocytes T. Les lymphocytes T exprimant un CAR reconnaîtront et détruiront les cellules tumorales exprimant l’antigène cible reconnu par l’ACm. L’immunothérapie adoptive basée sur les CAR autologues a déjà montré des résultats cliniques prometteurs en particulier dans les affections malignes à lymphocytes B (Porter et coll., N Engl J Med. 2011; 365[8]:725-733).

La deuxième approche, développée dans le cadre d’une collaboration avec Macrogenics, société américaine, utilise leur technologie « Dual-Affinity Re-Targeting [DART] » qui permet d’associer ciblage d’une cellule immunitaire à celui d’une cellule tumorale afin de créer une « synapse immunologique » spécifique de la cellule tumorale.

Ces quelques exemples montrent comment Servier tente de répondre aux besoins non satisfaits dans le traitement du cancer. Ces efforts permettent de constituer un portefeuille de candidats médicaments innovants associant petites molécules, anticorps monoclonaux synthétiques et thérapie cellulaire. Plus d’une dizaine d’entre eux sont en développement préclinique et clinique.

En outre, afin d’optimiser les chances de succès, un effort important de recherche translationnelle a été mis en œuvre avec l’objectif d’orienter le développement clinique sur la base d’éléments scientifiques.

**Conclusion**

Aujourd’hui, Servier, par un engagement fort, a l’ambition de développer au cours des prochaines années des traitements innovants contre le cancer qui auront un impact significatif et positif sur les patients atteints de cancer, ainsi que sur leur qualité de vie et celles de leurs familles.
Genomic medicine aims to identify molecular abnormalities that could act as therapeutic targets. This could ultimately result in subdividing a disease like breast cancer into multiple genomic entities, each defined by an oncogenic driver, whether mutation—as in the genes for phosphatidylinositol-4,5-bisphosphate 3 kinase, catalytic subunit α (PIK3CA), Akt strain-transforming RAC-alpha serine/threonine-protein kinase (AKT1), phosphatase and tensin homolog (PTEN), or receptor tyrosine-protein kinase erbB-2 (ERBB2, formerly HER2 or HER2/neu)—or amplification, as of ERBB2 or fibroblast growth factor receptor 1 (FGFR1). Targeting the oncogenic driver would then have the therapeutic effect of “oncogene deaddiction.” An additional aim is to understand how the presence of single or combined genomic alterations impairs susceptibility to targeted monotherapy via mechanisms responsible for nonresponse to conventional drug effect. Genomics can also be used to detect potentially lethal subclones causing treatment resistance, as well as to analyze DNA repair defects and mechanisms of immune response suppression. Applications such as these will give oncologists a better understanding of cancer biology at the personalized level. Ongoing studies are evaluating the clinical benefits of genomic medicine in breast cancer. A longer-term prospect is that of using genomics to decrypt the mutation process and block mutagenesis within the individual patient.

Recent progress in metastatic breast cancer management includes the development of second-generation receptor tyrosine-protein kinase erbB-2 (ERBB2 [formerly HER2 or HER2/neu]) inhibitors, first-generation mammalian target of rapamycin (mTOR) inhibitors, and new cytotoxic and hormone therapies. Yet survival rates remain depressingly low, despite such apparent progress. Innovative strategies for improving outcome include immunotherapy, epigenetic modulation, and, perhaps most notably, genomic medicine, which aims to identify molecular abnormalities that could act as treatment targets in a personalized approach. This strategy employs the classic tools of genomics such as Sanger sequencing and fluorescence in situ hybridization (FISH), but also multigene panel testing and the simultaneous identification of several hundred genes on new-generation high-throughput analyzers. Sequencing on this scale can identify mutations and copy-number variations ranging from a few genes to an entire genome, while array comparative genomic hybridization (CGH) using DNA chip technology can quantify the number of gene copies in individual samples.
Identifying breast cancer drivers at the personalized level

An oncogenic driver can be defined as a single or multiple alteration generated by an oncogene that causes a cancer to progress. The cancer is said to be in a state of oncogene addiction. Targeting the oncogenic driver should therefore have the therapeutic effect of oncogene deaddiction. Genomic analyses of primary and metastatic breast cancers have identified a great number of potential genomic drivers, prompting the concept that breast cancer could be subdivided into multiple genomic entities, each defined by its driver.

The historic breast cancer driver is ERBB2 amplification. Targeting ERBB2 with tyrosine kinase inhibitors increases objective response rates in patients positive for ERBB2 amplification. The most common drivers are mutations of phosphatidylinositol-4,5-bisphosphate 3 kinase, catalytic subunit α (PIK3Ca), but initial studies using nonselective phosphatidylinositol 3-kinase (PI3K) inhibitors have so far shown no evidence of oncogene deaddiction.

However, recent phase 1 data suggest that targeting PI3K with specific PI3Kα inhibitors produces an objective response in patients with PIK3CA mutations: response rates to the α-specific PI3K inhibitors BYL-719 (alpelisib) and GDC-0032 (taselisib) have been encouraging. Partial responses, in particular metabolic responses to GDC-0032, were observed in 73% of cases, confirming the role of PI3K as a breast cancer driver.

Studies are running on other genomic alterations in addition to PIK3CA. They include amplifications of cyclin D1 (CCND1), which are fairly common, but do not correlate with any increase in efficacy by cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors. Amplifications of fibroblast growth factor receptor 1 (FGFR1), however, found in around 10% of estrogen receptor-positive (ER+) breast cancers, may be associated with response to nonspecific FGFR inhibitors, including dovitinib and lucitanib.

More recently, much attention has focused on 2 less common genomic alterations: mutation in Ak strain-transforming RAC-α serine/threonine-protein kinase (AKT1, also known as protein kinase B (PDK1)), found in around 4% of breast cancers, that could define a subset of patients susceptible to AKT1 or mTOR inhibitors; and epidermal growth factor receptor (EGFR) amplification, seen in a subset of breast cancers triple-negative for estrogen, progesterone and ERBB2 receptors, that could define patients susceptible to EGFR inhibitors. As for the ERBB2 mutations found in 1%-2% of breast cancers, these could define susceptibility to ERBB2 inhibitors.

Other potential drivers have also been described, but their clinical relevance remains unknown due to a dearth of clinical trial data. They include suppression of phosphatase and tensin homolog (PTEN), loss of inositol polyphosphate-4-phosphatase type II protein (INPP4B), loss of cyclin-dependent kinase inhibitor 2A (CDKN2A), amplification of fibroblast growth factor receptor 2 (FGFR2), and mutations of Kirsten rat sarcoma viral oncogene homolog (K-ras), BRAF cell signaling protein, and serine/threonine kinase 11 (STK11).

Although multiple genomic alteration drivers have been proposed in breast cancer, we still have much to learn about how they may actually work. Three areas in particular are being studied to resolve this problem:

- developing and maintaining databases of cancer-related genes, then classifying the potential targets, so as to better identify the genomic alteration drivers.
- Tools need to be developed to determine whether a candidate driver is truncal (primary tumor-related) or subclonal (metastasis-related), since targeting a truncal event may be more effective (although this remains to be proved).
- determining whether the presence of single or combined genomic alterations impacts susceptibility to targeted monotherapy. For example, PIK3CA mutation has been reported as a factor for resistance to ERBB2 inhibitors in patients with ERBB2 amplification.
- determining whether oncogenic driver identification, man-
ly by DNA analysis, but also by gene expression deregulation and pathway activation in the absence of DNA mutation, can also provide information on susceptibility to target therapies. Thus mTOR pathway activation, defined by the presence of phosphorylated 4E-binding protein 1 (p4EBP1) or expression of its gene, indicates disease susceptibility to mTOR inhibitors, just as ER expression in early breast cancer is a relevant target in hormone-susceptible tissues that show but few DNA alterations.

When screening for multiple genomic alterations, the aim in sequencing the regions of interest with an intermediate depth of coverage is to identify drivers in an individual (or several individuals in parallel). Gene expression analysis and protein detection can be additional tools in identifying drivers unrelated to DNA alterations, such as ER expression and mTOR activation.

Defining secondary resistance mechanisms and subclonal drivers

Genomic tests are useful for identifying the mechanisms involved in the absence of response or the emergence of drug resistance. Estrogen receptor 1 (ESR1) mutations have been reported in 10% to 30% of metastatic ER+ breast cancers resistant to hormone therapy. It is interesting to note that very few tumors are ESR1-mutation positive in the early stages. This suggests that the mutation is either present at low levels in the primary tumor or is acquired on treatment exposure. To date no other mutation has been described in breast cancer with a level of evidence indicative of resistance to a specific targeted treatment.

Two different technologies can be used to monitor and kill the lethal clone. In this regard, we hypothesize that certain mutations are associated with treatment resistance even if the lethal clone is present in only a minority of cells at diagnosis: (i) in the primary tumor, or acquired on treatment exposure. To date no other mutation has been described in breast cancer with a level of evidence indicative of resistance to a specific targeted treatment.

In the scenario in which the resistant clone is present at low levels in the primary tumor or is acquired on treatment exposure, to date no other mutation has been described in breast cancer with a level of evidence indicative of resistance to a specific targeted treatment.

In the scenario in which the resistant clone is not detected in the primary tumor, but only during the subsequent disease course, circulating DNA could be used to monitor the development of lethal clones. Murtaza et al detected and characterized the emergence of a lethal clone during treatment by sequencing cancer exomes in plasma samples, identifying platelet-derived growth factor receptor-α (PDGFRα) mutation in a patient with trastuzumab resistance. Further research driven by the mining of massive long-term databases can be predicted to identify other mutations associated with poor response and/or resistance to standard adjuvant treatments in breast cancer.

In addition to the identification and targeting of resistant subclones, an important application of genomics is the quantification of intratumor heterogeneity, which in itself can be an indicator of poor prognosis, as in cancers generally. Several strategies are being developed. They include multiregion exome sequencing and copy-number analysis, circulating tumor-cell detection, and/or plasma-derived cell-free DNA sequencing. Recent studies point to intratumor heterogeneity in breast cancer. Although similar subclonal heterogeneity has been associated with treatment resistance in lung cancer, chronic lymphocytic leukemia, and colon cancer, its impact in breast cancer has yet to be proven.

Identifying the mechanisms of personalized tumor evolution

Mutations and DNA repair defects account for the genetic evolution of a tumor, while spatial and temporal differences in its evolution account for its internal heterogeneity. As well as identifying lethal and driver subclones, genomics has 3 further potential applications in this area:

- identifying DNA repair defects,
- quantifying genome instability and intratumor heterogeneity,
- decrypting mutation mechanisms.

Identifying DNA repair defects at the individual level could lead to developing personalized synthetic lethality strategies. For example, inhibitors of poly(ADP-ribose) polymerase (PARP) have proved useful in patients with breast cancer 1 and 2, early-onset (BRCA1/2) mutations. Other mutations in DNA repair genes, such as ataxia-telangiectasia mutated kinase (ATM), ATM and Rad3-related protein (ATR), and DNA excision repair protein 1 (ERCC1), could also be targeted by this approach. In addition, DNA repair defects lead to genome instability, for which signatures have been developed. Such signatures can be specific to a DNA repair pathway or more general, and are identified by whole-exome sequencing on an instrument such as the Affymetrix Genome-Wide SNP Array 6.0 platform that detects defects in homologous repair (HR) and mismatch repair (MMR). HR-defect signatures could predict DNA susceptibility to alkylating agents and PARP inhibitors in breast cancer patients.

More recently, accumulation of genome alterations suggestive of genome instability has been associated with everolimus resistance. In the longer term, genomics could also be used to identify mutation mechanisms, such as overexpression of apolipoprotein B messenger RNA (mRNA) editing enzyme, catalytic polypeptide-like (APOBEC), thereby permitting personalized blockade of the mutagenesis process.

Identifying the mechanisms of immune system impact on treatment response

Morphological and molecular pathology, specifically the quantification of stromal tumor infiltrating lymphocytes, has shown...
the immune system to be involved in breast cancer progression and treatment response.31-33 Genomics can extend the insights gained from morphology by decrypting the molecular mechanisms enabling disease to circumvent the immune system in particular individuals and accounting for the immune system impact on drug treatment response.

Recent data suggest that certain missense somatic mutations generate proteins (neoantigens) that may be recognized by the host immune system and subsequently induce an antitumor immune response. Proof of concept in silico research found a positive correlation between the presence of immunogenic variants, increased amounts of cluster of differentiation 8a (CD8A) mRNA, and very good results in terms of antitumor immune response in many types of solid tumors.34 Elevated expression of antitumor cytotoxic T-cell exhaustion markers (programmed death-ligand 1 [PD-L1] and cytotoxic T-lymphocyte antigen 4 [CTLA4]), determined by RNA sequencing, have also been observed in conjunction with immunogenic variants, suggesting that these checkpoints were induced following an activated T-cell response. Recent mechanistic studies using mice melanoma models have borne out this concept.35 In human melanoma samples, the presence of a neoantigen signature was associated with a stronger and more durable response to the CTLA-4 antibody ipilimumab.36 If this concept can be extrapolated to breast cancer, certain mutations could also predict response to immunotherapy, such as tumor-cell inhibition at the immune (T cell) checkpoint. This hypothesis suggests that determining the spectrum of immunogenic somatic mutations in breast cancer may be important for identifying biomarkers and developing drugs in immunotherapy.

In contrast to the intrinsic cell mechanism of pathway activation at the T-cell checkpoint, oncogene addiction induces immunosuppressive mechanisms in the microenvironment.37 EGFR-driven lung cancers have been shown to upregulate the programmed cell death protein 1 (PD-1)/PD-L1 pathway.38 Similarly, in gastrointestinal stromal tumors, imatinib mesylate treatment decreased tumor-cell production of the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO).39 In these 2 examples, tumor-cell inhibition and T-cell checkpoint blockade combined synergistically with targeted therapy. These data suggest a paradigm of target inhibition combined with immunostimulation for oncogene-addicted tumors. In tumors overexpressing ERBB2, both innate and adaptive immunity certainly play an important role in the efficacy of trastuzumab. But it can be argued that combining cytotoxic chemotherapy with trastuzumab offers both signal inhibition and immune activation, and is already successful in terms of survival, with figures now exceeding 90% in stage I/II disease.40 Thus, future breast cancer genomics must not be considered independently of the immune response and there will be much to learn from integrating the evaluation of genomic drivers with the measurement of lymphocyte infiltration.

Conclusion and future prospects
The many potential applications of genomics for improving results in metastatic breast cancer include identifying the driver(s) involved, predicting resistance, decrypting DNA repair defects, and avoiding tumor-induced immune suppression. These applications should provide oncologists with a better understanding of cancer biology at the personalized level. Ongoing studies are seeking to determine the clinical usefulness of the genomic approach and hopefully demonstrate that genomic medicine improves results in breast cancer patients.

The future promises further prospects for genomic medicine in breast cancer treatment:
- new bioinformatics tools for personalized target identification;
- ever more sensitive and specific protein assays to improve target characterization;
- rapid translation of research insights into the clinic to inform preoperative management;
- and, perhaps the greatest challenge, the identification of germline mutations or polymorphisms associated with increased metastatic risk. ■

References
La médecine génomique a pour but d’analyser des anomalies moléculaires qui pourraient devenir utiles pour le traitement des cancers. La médecine génomique a pour but d’analyser des anomalies moléculaires qui pourraient devenir utiles pour le traitement des cancers. Elle permettrait ainsi de cibler le traitement des cancers et de prédire l’efficacité du traitement.

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Keywords: cancer; personalized medicine; genomics; activating mutation; oncogenic driver; subclone; T-cell checkpoint
Agents targeting the fibroblast growth factor receptor (FGFR) pathway have shown promising results to date, and further trials are warranted to better define the role of these agents in breast cancer and potential predictive factors of response.

Despite the important results of research to date, metastatic breast cancer (MBC) remains an incurable disease, therefore the research of new targets and targeted therapies is needed. Hormone receptors and human epidermal growth factor 2 (HER2) hyperexpression represent current drug targets in the management of MBC. Specific treatments against these targets enable a greater disease control rate and an increase in overall survival. Furthermore, recent evidence demonstrated that a multitarget approach is more effective than monotherapy. For example, in hormone receptor-positive MBC the association of hormone therapy with mTOR (mammalian target of rapamycin) inhibitors is superior to hormone therapy alone. In HER2-positive breast cancer the association of pertuzumab and trastuzumab is superior to trastuzumab alone. Fibroblast growth factor (FGF) and its pathway are involved in tumor growth, proliferation, invasion, and angiogenesis. It therefore represents an interesting new target for cancer treatment, particularly fibroblast growth factor receptor 1 (FGFR1), which is amplified in about 10% of cases of MBC expressing estrogen receptors (ER). This aberration is associated with poor prognosis. Several molecules targeting the FGF-FGFR pathway are under investigation, and two phase 1/2 studies have already demonstrated the efficacy of tyrosine kinase inhibitors in luminal B tumors. Further studies are needed to confirm these preliminary results, identify which molecules inhibit the FGFR pathway most effectively, and determine if these molecules should be administered in association with other targeted therapies. The results of ongoing studies will help to define the importance of the FGF-FGFR pathway as a new target in MBC.

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Emergent targets in metastatic breast cancer

by E. Zanardi, G. V. Bianchi, and F. De Braud, Italy

Metastatic breast cancer (MBC) accounts for about 1 million new cases of cancer each year worldwide. Many systemic treatment options are available for advanced breast cancer, including endocrine therapy, chemotherapy, and anti-human epidermal growth factor 2 (HER2) molecules, but it remains the most common cause of cancer death in women. In recent years, the median survival of patients affected by breast cancer has dramatically improved, in particular in HER2-positive breast cancer and estrogen receptor (ER)-positive tumors, due to new, approved targeted therapies. In 1998, trastuzumab was approved by the US Food and Drug Administration (FDA) for the treatment of MBC, completely changing the prognosis and survival of patients in the metastatic setting. In a mono-institutional retrospective analysis in 2010, Dawood et al observed that
**Figure 1.** Interaction between cyclin D1 and CDK (cyclin-dependent kinase) 4/6 represents the key passage from the G1 to the S phase in the cell cycle.

Several mitogenic signals converge at the level of this complex. The interaction of CDK 4/6 with cyclin D1 is responsible for hyperphosphorylation of retinoblastoma (RB) tumor suppressor proteins; this results in pRB inhibition and release of E2F transcription factors and transcriptional regulation of genes determinant in G1/S transition and cell cycle progression through the restriction point (lightning bolt). Abbreviations: Akt, protein kinase B; AR, androgen receptor; ER, estrogen receptor; G0, gap 0 (resting); G1, gap 1; G2, gap 2; M, mitosis; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PR, progesterone receptor; S, synthesis; STAT, signal transducer and activator of transcription; Wnt, Wingless-related integration site.


HER2-positive MBC patients treated with trastuzumab have a survival rate similar to that of MBC patients with HER2-negative disease, independent of the expression of hormone receptor. Recently, the results of the CLEOPATRA (CLinical Evaluation Of Pertuzumab And TRAstuzumab) and trial have further improved the prognosis of these breast cancer subtypes, demonstrating that the combination of two HER2 monoclonal antibodies (trastuzumab and pertuzumab) with docetaxel prolongs progression-free survival (PFS) and overall survival (OS) in comparison with the combination of trastuzumab and docetaxel. In fact, median PFS was 18.5 months in the pertuzumab arm versus 12.4 months in the control arm (hazard ratio [HR], 0.62; 95% confidence interval [CI], 0.51-0.75; P<0.001); median OS was 56.5 months in the pertuzumab group versus 40.8 months in the control group (HR, 0.68; 95% CI, 0.56-0.84; P<0.001). In ER-positive HER2-negative MBC, everolimus was the first targeted therapy associated with hormonal treatment demonstrating a better PFS than endocrine therapy alone. Unfortunately, in triple-negative breast cancer (TNBC) no targeted therapies have been identified to date; chemo therapeutic options remain the standard of care, with dismal impact on survival for these patients. The expression of receptors of endothelial growth factor receptor and androgen are under investigation as possible targets in TNBC. Despite targeted therapies changing the scenario of breast cancer treatment, this disease remains incurable in the metastatic setting. The treatment of metastatic disease, therefore, remains a clinically meaningful unmet need, and many preclinical and clinical studies are in progress in order to identify new molecular targets, their relevance in tumor progression, and their potential role as anticancer drugs.

Currently emerging new targets such as cyclin-dependent kinases (CDK) and phosphoinositide 3-kinase (PI3K) pathways are the most widely studied mechanisms implicated in both primary and secondary endocrine resistance. Cell cycle–related genes and proteins are frequently deregulated in breast cancer. The progression from the G1 to S phase is a key checkpoint during cellular replication, and the fundamental step in this process is the interaction between CDK and cyclin proteins. The primary target of CDK action is the retinoblastoma protein (pRB) in a phosphorylation process that leads to the release of transcription factors of the E2 promoter-binding-protein-dimerization partners (E2F-DP) family, permitting phase S entry (Figure 1).
Approximately 15% to 20% of human breast cancers exhibit amplification of the cyclin D1 gene (CCND1), and a higher proportion of tumors overexpress CCND1 protein. Many CDK4-6 inhibitors are under evaluation in clinical trials. The FDA recently approved palbociclib (Ibrance®, Pfizer), an inhibitor of CDK 4 and 6, in combination with letrozole for the treatment of postmenopausal women with ER-positive, HER2-negative advanced breast cancer, as a first-line endocrine therapy in metastatic disease. This accelerated approval was based on the results of a randomized multicenter phase 2 trial, in which the combination of palbociclib and letrozole improved PFS versus letrozole alone, in postmenopausal women with ER-positive, HER2-negative MBC who had not received previous systemic treatment for advanced disease.

The PI3K/Akt (protein kinase B)/mTOR (mammalian target of rapamycin) pathway is an intracellular pathway that leads to cell growth and tumor proliferation. This pathway is associated with resistance to endocrine therapy, HER2-directed therapy, and cytotoxic therapy in breast cancer. It is currently the only compound approved for the treatment of hormone receptor–positive, HER2-negative metastatic or locally advanced breast cancer, as previously described.

Buparlisib is an investigational oral pan-PI3K inhibitor that targets the four isoforms of class I PI3K (α, β, γ, δ). It has been widely studied in phase 2 and 3 trials in combination with endocrine therapies or with chemotherapy. Other compounds, such as GDC-0941 and BEZ235, are being evaluated in phase 1 and 2 clinical trials. The next promising target in breast cancer is the fibroblast growth factor (FGF) pathway, which will be discussed in the subsequent paragraphs.

**FGF-FGFR signaling pathway**

FGFs and their receptors, known as fibroblast growth factor receptors (FGFRs), play an important role in cancer pathogenesis. The effects of activation of this pathway not only concern tumor cells, but also surrounding stroma, and are involved in cancer cell growth, survival, migration, and angiogenesis. The FGF family comprises 18 small molecules residing mainly in the extracellular matrix. These ligands exert their effect by binding with four tyrosine kinase receptors (FGFR1, FGFR2, FGFR3, FGFR4); a fifth receptor (FGFR5) has no tyrosine kinase domain; and an intracellular portion with a tyrosine kinase domain.

FGFRs are transmembrane receptors composed of three parts: an extracellular domain containing three immunoglobulin (Ig)-like fragments, a single-pass transmembrane domain, and an intracellular portion with a tyrosine kinase domain and a carboxylic acid tail. The binding between FGFRs with the receptor determines FGFR dimerization and the activation of intracellular signaling through the process of tyrosine phosphorylation. This involves multiple pathways, which include the mitogen-activated protein kinase (MAPKs), PI3K, phospholipase Cγ (PLCγ), and PKC, and signal transduction and activation of transcription (STATs) pathways.
nal transducers and activators of transcription (STATs) pathways (Figure 2). Several feedback inhibitors of the FGF pathway, including members of the Sprouty (Spry) family and Sef (similar expression to FGF), have been identified. These inhibitors interfere at different steps of the pathway to block intracellular signaling. The downregulation of feedback inhibitors in association with an increased expression of FGF ligands through autocrine or paracrine production, and mutations/amplification in FGFR, results in tumor cell proliferation, progression, metastasis, and angiogenesis.14,15

FGFR’s aberration in breast cancer

All four FGFRs have been studied in breast cancer to evaluate a possible correlation between breast cancer and deregulation of FGFR activity. Several studies report amplification of FGFR1 in about 10% of breast cancers. Evidence of a correlation between this amplification and biological markers, such as hormone receptors or HER2, is inconsistent, but evidence indicates that a correlation between FGFR1 amplification and worsening of breast cancer prognosis exists. ER-positive patients with FGFR1 amplification are more likely to develop metastases and have significantly shorter OS, independent of other prognostic factors such as tumor size, lymph node invasion, and grading. The prognostic correlation was observed only in ER-positive tumors, suggesting a negative interaction between FGFR1 and ER signaling that results in a poor prognosis.16 Moreover, Turner et al observed that FGFR1 amplification drives resistance to endocrine therapy in vitro and that FGFR1 amplification occurs more frequently in progesterone receptor (PR)–negative tumors. Loss of PR expression could reflect activation of FGFR signaling, and it is thought to be a biomarker of FGFR1 activity in breast cancer proliferation. This evidence suggests that FGFR1 amplification is one of the major drivers of luminal B breast cancer, which is associated with poor prognosis.17 Amplification of FGFR2 is observed in about 4% of TNBCs, while no amplification was found in the other subtypes.

No breast cancer–related amplification was observed with FGFR3, although FGFR3 could have a role in resistance to tamoxifen. Increased levels of FGFR3 protein were found in a group of patients that did not respond to treatment with tamoxifen.18 FGFR4 amplification was found in 10% of breast cancers in a small study, and they were associated with positive ER and PR status. In a retrospective analysis of ER-positive breast cancers treated with tamoxifen in a metastatic setting, high levels of FGFR4 were associated with poor clinical benefit and shorter PFS, suggesting a relationship between FGFR4 expression and tamoxifen failure.19 It is noteworthy that FGFR4 is one of the HER2-enriched specific genes included in the 50-gene intrinsic subtype predictor (PAM50),20 but the significance of FGFR4 activation in HER2-positive breast cancer is still unknown.21 The genomic aberrations described above lead to constitutive receptor activation responsible for cancer growth.15

FGF pathway and angiogenesis

FGFs are among the first angiogenic factors described. They regulate endothelial cells inducing proliferation, migration, and differentiation of endothelial cells, and creating a favorable environment for vasculature growth. Angiogenesis is a crucial point for tumor proliferation and metastatic diffusion, and FGFs play a central role by promoting cell growth in endothelial cells expressing FGFR. The main FGFR expressed by endothelial cells is FGFR1, but FGFR2 is also present in small amounts. The binding of FGF to FGFR stimulates new vessel formation and maturation by inducing endothelial cell proliferation, favoring extracellular matrix degradation, and altering intracellular adhesion.22

Other important regulators of angiogenesis are the vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs). Intimate crosstalk is thought to occur between VEGF/VEGFR and FGF pathways. In fact, FGF and VEGF synergistically induce vascularization, but each has distinct ef-

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**Figure 3.** Schematic representation of fibroblast growth factor (FGF) effects triggered in endothelial cells to induce neo-angiogenesis.

flicts on tumor survival and vessel functionality; VEGF acts at the beginning of angiogenesis, whereas FGF stimulates vessel growth in both early and late angiogenesis (Figure 3, page 251).22

Since angiogenesis is one of the milestones for the development and progression of tumors, the study of this process is very important for the definition of new therapeutic strategies. In fact, the introduction of angiogenesis inhibitors has changed, in the last ten years, the natural history of locally advanced and metastatic renal cell carcinoma.23,24 However, clinical trials with these molecules in MBC did not produce the same results.25,26 In particular, treatment with bevacizumab, an anti-VEGF monoclonal antibody, in association with paclitaxel, resulted in a statistically significant increase in PFS compared with chemotherapy alone,27-29 but no significant difference in OS.30 For this reason, the FDA did not approve bevacizumab plus paclitaxel for the treatment of MBC.31 Proportional PFS in patients with MBC, in association with bevacizumab, was associated with a statistically significant increase in OS compared with chemotherapy alone,27-29 but no significant difference in OS.30 For this reason, the FDA did not approve bevacizumab plus paclitaxel for the treatment of MBC.31

The FGF pathway as an emergent treatment target

As the FGFR signaling pathway may be a cause of breast cancer, and related to poor prognosis,16 it represents an important target for the treatment of breast cancer. In particular, treatment with bevacizumab, an anti-VEGF monoclonal antibody, in association with paclitaxel, resulted in a statistically significant increase in PFS compared with chemotherapy alone,27-29 but no significant difference in OS.30 For this reason, the FDA did not approve bevacizumab plus paclitaxel for the treatment of MBC.31 Prolongation of PFS with bevacizumab treatment highlights the importance of angiogenesis in tumor progression. However, the lack of OS benefit implies that the optimal way to inhibit this process or the right population in whom this drug should be used remains to be discovered. Considering that the VEGF and FGF pathways interact with each other in hyperangiogenesis,17 disorganization of primitive tumor vasculature, it is thought that targeting both pathways may be more efficient than targeting one pathway alone at controlling cell proliferation and metastatic diffusion.

The first reported trial with a specific FGFR inhibitor evaluated dovitinib in metastatic breast cancer. André and colleagues suggested that dovitinib could have antitumor activity in FGF-amplified tumors, but not in FGF-nonamplified tumors. In FGF1-amplified tumors, with amplification detected not only by silver-enhanced in situ hybridization (SISH), but also with quantitative (real time) polymerase chain reaction (qPCR), a reduction in tumor size of up to 20% was observed. Furthermore, preclinical trials suggest that dovitinib is able to reverse endocrine resistance.17 These data indicate that dovitinib could be an important treatment in breast cancer, not only as a single agent, but in combination with other therapies, such as endocrine therapy.31

Another important, recently published phase 1/2 trial evaluated the safety and efficacy of lucitinib in solid tumors. Lucitanib is a potent, highly selective inhibitor of the tyrosine kinase activity of FGFR types 1 and 2, VEGFR types 1 to 3, and PDGFR types α and β, which are essential kinases for tumor growth, survival, migration, and angiogenesis. This study started with a dose-escalation phase, in which the maximum tolerated dose (MTD) and the recommended dose (RD) were identified. MTD was 30 mg lucitanib once daily and RD for the next phase was 20 mg once daily, although this was subsequently adjusted to 15 mg because more than half of patients required dose reductions. The efficacy of lucitanib was in particular observed in FGF-aberrant breast cancers, for which the disease control rate was 100% (six patients with partial response and six with stable disease). In angiogenesis-sensitive patients, lucitanib is a potent inhibitor of FGFR and VEGFR, and it is probable that the double blockage of these pathways could explain the notable efficacy of lucitanib in FGFR1-amplified breast cancers.34 This contrasts with the limited activity of single inhibitors of VEGF or FGFR in breast cancers.35,36 Looking at the comparison between these

<table>
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Table I. Half maximal inhibitory concentration (IC50) of different nonselective multikinase inhibitors.

Multiprotein inhibitors currently being evaluated (yellow squares) are compared with molecules that have already been approved in cancer treatment (red squares).

Abbreviations: FGFR, fibroblast growth factor receptor; PDGFR, platelet-derived growth factor receptor; unk, unknown; VEGFR, vascular endothelial growth factor receptor.
nonselective multikinase inhibitors and those studied to date (for example, sunitinib, pazopanib, and axitinib, etc), the former have an IC50 (half maximal inhibitory concentration) inferior to the latter, suggesting that the former are more effective at inhibiting kinases (Table I). The spectrum of activity of lucitanib appears consistent, with clinical benefit in both FGFR-aberrant and angiogenesis-sensitive populations.

There are several ongoing phase 1 trials with selective TKIs, such as BGJ398, AZD4547, LY2874455, and JNJ-42756493. These TKIs in vitro are very potent inhibitors of FGFR1, FGFR2, and FGFR3. Preliminary results of phase 1 trials with AZD4547 and BGJ398 don’t show a significant activity in breast cancers, but final data are still expected as well as the evaluation of these TKIs in association with hormone therapy (NCT01202591). Several mAbs against FGFR are in preclinical development: they can be highly specific for FGF ligand or FGFR isoform, and they are able to recruit the immune system via antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity, increasing antitumor activity. Until now no mAbs against FGFR have demonstrated activity in breast cancer and toxicity represents an important limit in evaluating this class of therapies. Future trials are needed to evaluate efficacy of this treatment strategy.

Another approach is the use of ligand traps, such as FP-1039, which consist of a modified extracellular domain of FGFR1 fused to the crystalizable fragment region of human immunoglobulin. It is thought that FP-1039 is able to sequester multiple FGF ligands, causing antiangiogenic and antitumor effects, as shown in preclinical in vivo studies. Unfortunately, a phase 2 study testing FP-1039 in endometrial cancer was deemed unfeasible because none of the 70 patients screened qualified, and no data are available for breast cancers.

The development of these treatment strategies for the FGFR pathway (Table II) demonstrates its importance in the cancer process. In breast cancer, FGFR1 amplification is thought to be the most important FGFR aberration responsible for tumor growth and progression. Therefore, the selection of patients with FGFR1 amplification probably represents the first step in identifying breast cancers that can benefit from target treatments of the FGFR pathway. FGFR1-amplified breast cancers are associated with poor prognosis, so developing a treatment that is able to improve prognosis of patients with these kind of tumors would be valuable. The first step is to find the most appropriate patients for this type of therapy by identifying, using SISH or qPCR, patients with FGFR1-amplified breast cancer. The second step is to identify the most effective types of treatment among those that are in development. Currently, nonselective multikinase inhibitors appear to be the most active treatment against FGFR1-amplified breast cancers, but these data need to be confirmed. Furthermore, it is important to identify if TKIs should be used in monotherapy or in association with hormone therapy or chemotherapies. Data mentioned above demonstrate that FGFR1 amplification is related to endocrine resistance, so inhibition of this pathway could improve sensitivity to endocrine treatment. For this reason, the association of hormone therapy with FGFR inhibitors could be an efficient strategy. Positive responses at all these steps could lead to the definition of a new biological marker in breast cancer, as is the case with HER2, which would change the prognosis in a subgroup of our patients.

### Conclusion

At present, the identification of a target responsible for tumor cell proliferation, survival, and migration is a crucial goal in the development of a new treatment strategy. Hormone receptors in breast cancer were the first target in cancer treatment, until the delineation of the role of HER2 protein, which dramat-

<table>
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</table>

Emergent targets in metastatic breast cancer – Zanardi and others
ically changed the treatment and prognosis in the 25% of breast cancers that are HER2 positive. In fact, the use of mAbs and TKIs that target the HER2 pathway have been shown to be effective in the neoadjuvant, adjuvant, and metastatic settings, which has led to significant increases in pathological complete response, PFS, and OS. 40-42 Recent evidence has shown that agents targeting HER2 when used in association are more effective than monotherapy.4,43,44 Many other intracellular mechanisms of tumor growth are well known, and targeted therapies against different steps of cell proliferation are emerging. The PI3K/Akt/mTOR pathways and CDK pathways are crucial in tumor progression, and their blockade has promising efficacy in MBC. The FGF pathway, and in particular FGFR1 amplification, could also represent a new fundamental target in breast cancer management. FGFR1 seems to be responsible for endocrine resistance, so the association of hormone therapy with FGF-targeted therapy could be an effective strategy to overcome endocrine resistance and define a specific role for these agents. Also, the crucial role that FGF pathway plays in the angiogenic process is indicative of the importance that this new therapeutic target could represent. Several studies are warranted to define the most effective agents in FGFR-aberrant breast cancer and the best combinations to enhance antitumor activity. Moreover, predictive biomarkers are needed to facilitate the selection of the right population for these treatments, thus maximizing patient benefit. A further approach will be to act at different stages of these pathways to completely block mechanisms of tumor progression and avoid alternative ways for tumor cells to proliferate.

References

CIBLES ÉMERGENTES DANS LE TRAITEMENT DU CANCER DU SEIN MÉTASTATIQUE

Malgré des résultats de recherche importants à ce jour, le cancer du sein métastatique (CSM) reste une maladie incurable, la recherche de nouvelles cibles et de traitements ciblés est donc nécessaire. Les récepteurs hormonaux et l’hyperexpression du récepteur 2 du facteur croissance épidémique humain (HER2) sont des cibles médicamenteuses actuelles dans la prise en charge du CSM. Des traitements spécifiques contre ces cibles permettent un meilleur taux de contrôle de la maladie et une augmentation de la survie globale. De plus, les polythérapies aux cibles multiples ont récemment démontré une meilleure efficacité que les monothérapies. Par exemple, dans le CSM aux récepteurs hormonaux positifs, l’association d’une monothérapie hormonale avec des inhibiteurs de la protéine mTOR (cible de la rapamycine chez les mammifères) est plus efficace que la monothérapie hormonale seule. Dans le cancer du sein HER2-positif, l’association du pertuzumab et du trastuzumab est plus efficace que n’importe quel anticorps monoclonal HER2 seul en termes de résultats. Le facteur de croissance fibroblastique (FGF) et ses voies sont impliqués dans la croissance, la prolifération, l’invagination et l’angiogenèse tumorales et représentent donc une nouvelle cible intéressante pour le traitement du cancer. Le FGFR1 (récepteur 1 au facteur de croissance fibroblastique) en particulier est augmenté dans environ 10 % des cas de CSM exprimant le récepteur aux estrogènes (RE) et cette mutation est associée à un mauvais pronostic. Plusieurs molécules ciblant les voies FGF–FGFR sont en cours de recherche et deux études de phase 1/2 ont déjà démontré l’efficacité des inhibiteurs de la tyrosine kinase dans les tumeurs de type luminal. D’autres études sont nécessaires pour confirmer ces résultats préliminaires, identifier quelles molécules inhibent le plus efficacement la voie FGFR et pour savoir si elles pourraient être associées à d’autres traitements ciblés. Les résultats des études en cours nous aideront à définir l’importance de la voie FGF–FGFR comme nouvelle cible dans le CSM.
Lung cancer is the leading cause of cancer death worldwide. Non–small cell lung cancer accounts for an estimated 80%-85% of lung cancers and can be divided into 2 predominant types, adenocarcinoma (~50% of cases) and squamous cell carcinoma (~30% of cases). All histological types of lung carcinoma are associated with smoking, the association being strongest for squamous and small-cell lung carcinoma. Squamous cell carcinoma tumors rarely harbor mutations in epidermal growth factor receptor (EGFR) or anaplastic lymphoma receptor tyrosine kinase (ALK) fusions. Targeted therapies that are effective against adenocarcinoma are ineffective or contraindicated in squamous cell carcinoma, for which treatment options are limited. Recently, molecular profiling of squamous cell carcinoma of the lung revealed targetable alterations, among them, fibroblast growth factor receptor (FGFR) amplifications, discoidin domain receptor tyrosine kinase 2 (DDR2) mutations, and phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α (PIK3CA) mutations/amplifications. At present, a number of clinical trials with FGFR inhibitors are ongoing in squamous cell carcinoma patients. Furthermore, exploration of immune checkpoints has resulted in novel immunotherapies designed to interrupt signaling through the programmed cell death protein 1 pathway in lymphocytes. Modulation of this pathway can restore antitumor immune responses and preliminary evidence clearly shows its activity in squamous cell carcinoma of the lung. This review briefly presents the standard treatment options available now for patients with stage IV squamous cell carcinoma and current knowledge of molecular alterations in this subgroup, focusing in particular on alterations with real potential to be therapeutically targeted.

**Epidemiology of squamous cell carcinoma of the lung**

Squamous cell carcinoma of the lung is the second most common lung cancer subtype. It is closely linked to smoking habit. Its pathogenesis seems to be different from that of adenocarcinoma. Well-differentiated squamous cell carcinoma characteristics in tissue show keratinization, intercellular bridges, and pearl formation (Figure 1). On immunohistochemical examination, p63 is found to be positive and thyroid transcription factor 1 (TTF-1) is negative.

The sequential pathogenesis is well described: bronchial epithelial cells exposed to smoking over an extensive period develop basal cell hyperplasia, squamous metaplasia, carcinoma in situ and, finally, squamous cell carcinoma.
Squamous cell carcinoma of the lung is a heterogeneous entity. In a recent study, squamous cell carcinoma tumors were subclassified according to messenger RNA (mRNA) expression subtypes. That study shows that basaloid squamous cell carcinoma is a distinct histomolecular entity, allowing it to be recognized and distinguished from the non-basaloid type.

Treatment for stage IV squamous cell carcinoma of the lung

In advanced non–small cell lung cancer (NSCLC), histological subtyping has become an essential consideration in guiding treatment decision-making. For NSCLC patients with tumors harboring epidermal growth factor receptor (EGFR) mutation or anaplastic lymphoma receptor tyrosine kinase (ALK) rearrangement, treatment with an EGFR tyrosine kinase inhibitor (eg, gefitinib, erlotinib, or afatinib) or ALK inhibitor (eg, crizotinib), respectively, is recommended. The presence of EGFR mutations and ALK rearrangements is clearly related to adenocarcinoma subtype. The majority of guidelines suggest that there is no established need for EGFR or ALK testing in those patients with confident diagnosis of squamous cell carcinoma by the pathologist. Bevacizumab is contraindicated in patients with histological features of squamous cell carcinoma since a relevant rate of severe pulmonary hemorrhage has been reported with its use in this group of patients. Pemetrexed is also contraindicated in this group of patients because of worse survival outcomes reported for the cisplatin/pemetrexed combination when compared with the cisplatin/gemcitabine combination. For those reasons, in patients with stage IV squamous cell carcinoma, the standard treatment remains a platinum doublet without pemetrexed or bevacizumab.

In recent studies using new compounds, some encouraging findings have been reported in patients with histological features of squamous cell carcinoma. In advanced NSCLC patients, the contribution of nab-paclitaxel, a 130-nm albumin-bound (“nab”) form of paclitaxel designed to use endogenous albumin pathways to increase intratumoral concentrations of the active compound, has been analyzed. In a randomized trial, nab-paclitaxel achieved a higher response rate compared with paclitaxel when both were used in combination with carboplatin in the first-line treatment of NSCLC. In that trial, in the subgroup of patients with squamous cell carcinoma, nab-paclitaxel/carboplatin achieved an overall response rate of 41% compared with 24% for paclitaxel/carboplatin. The molecular mechanisms explaining the antitumor activity of nab-paclitaxel/carboplatin in patients with squamous cell carcinoma remain unknown.

Patients with treatment-naive advanced squamous cell carcinoma and good performance status generally receive a platinum-based doublet as standard therapy. Despite numerous clinical trials, the standard of care has remained the same for several decades. Necitumumab, a human anti-EGFR monoclonal antibody, has recently been analyzed in advanced squamous cell carcinoma patients in a phase 3 trial comparing cisplatin/gemcitabine with and without necitumumab (n=1093). Patients assigned to the necitumumab arm, compared with the platinum-based-therapy alone arm had a statistically significant improvement in overall survival (median overall survival: 11.5 months for the cisplatin/gemcitabine/necitumumab arm versus 9.9 months for the cisplatin/gemcitabine arm; hazard ratio [HR] 0.84; P=0.0012).

In the second-line setting, ramucirumab, a monoclonal antibody against vascular endothelial growth factor receptor 2 (VEGFR2), has been analyzed in a phase 3 trial comparing docetaxel with ramucirumab or placebo in patients who had progressed after platinum-based therapy (n=1253). Patients with NSCLC of any histological type were eligible, and approximately 25% of patients enrolled had squamous NSCLC. Patients assigned to docetaxel and ramucirumab, compared with docetaxel and placebo, experienced a statistically sig-
significant improvement in overall survival (median overall survival: 10.5 months for the docetaxel/ramucirumab arm versus 9.1 months for the docetaxel/placebo arm; HR 0.86; \( P=0.023 \)).

In the LUX-Lung 8 study (not an acronym), patients with squamous cell carcinoma following failure of first-line chemotherapy were randomized to receive afatinib or erlotinib. Median progression-free survival (PFS) was significantly higher for afatinib than erlotinib, both by independent central review (2.4 versus 1.9 months; \( P=0.0427 \)) and by investigator review (2.7 versus 1.9 months; \( P=0.0053 \)). Overall survival results from this study are pending.\(^6\)

Promising results have also been observed with anti–programmed cell death protein 1 (PD-1) and anti–programmed death-ligand 1 (PD-L1) strategies, but these will not be discussed here.

In summary, the standard treatment for patients with stage IV squamous cell carcinoma who have good performance status remains a platinum doublet. In first-line treatment, recent studies have shown positive results with nab-paclitaxel/caboplatin and with the necitumumab/cisplatin/gemcitabine combination. In previously treated patients, randomized trials have shown positive results with the ramucirumab/docetaxel combination and with afatinib. These findings together with results achieved using anti–PD-1 and anti–PD-L1 strategies will change the treatment algorithm in patients with squamous cell carcinoma.

### Molecular alterations in squamous cell carcinoma of the lung

In recent years, knowledge of the molecular pathogenesis of NSCLC has increased remarkably and brought about changes in the principles of treatment. However, these changes have been mainly limited to adenocarcinoma of the lung. Molecular genotyping of adenocarcinoma is currently the standard of care and includes analysis of EGFR and ALK, which are altered in approximately 20% of adenocarcinoma patients, for whom there are approved targeted therapies.

Several studies have attempted to distinguish lung adenocarcinoma from squamous cell carcinoma at the molecular level and, recently, genetic alterations in squamous cell carcinoma have been described. The most prevalent mutations found in lung cancer are those within the tumor protein 53 gene (TP53). In addition to TP53 mutation, adenocarcinoma tumors often contain loss-of-function mutations in other tumor suppressor genes, such as serine/threonine kinase 11 (LKB1/STK11), neurofibromin 1 (NF1), cyclin-dependent kinase inhibitor 2A (CDKN2A), SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4 (SMARCA4), and kelch-like ECH-associated protein 1 (KEAP1).\(^7\) Squamous cell carcinoma tumors have a different spectrum of inactivated tumor suppressors, which partly overlap with those seen in adenocarcinoma tumors: TP53 mutation is observed in the majority of squamous cell carcinoma tumors and other mutations can include inactivation of CDKN2A, phosphatase and tensin homolog (PTEN), KEAP1, MLL2 (also known as KMT2D), [lysine] (K)-specific methyltransferase 2D, major histocompatibility complex, class I, A (HLA-A), nuclear factor, erythroid 2-like 2 (NFE2L2), Notch 1 (NOTCH1), and retinoblastoma 1 (RB1).\(^8,9\)

A large next-generation sequencing (NGS) analysis of 178 squamous cell carcinoma tumors performed by the Cancer Genome Atlas Research Network identified a mean of 360 exome mutations, 323 altered copy number segments, and 165 genomic rearrangements per tumor.\(^10\) The mean somatic mutation rate of 8.1 mutations per megabase observed in this squamous cell carcinoma study was higher than that previously reported for a number of other cancer types. The spectrum of somatic copy number alteration in squamous cell carcinoma was mostly similar to that found in adenocarcinoma with the notable exception of selective amplification of a region on chromosome 3q. The evidence of a unique clinical and pathologic character of squamous cell carcinoma was confirmed in this study, which identified SRY (sex determining region Y)-box 2 (SOX2) amplification, NFE2L2 and KEAP1 mutations, phosphatidylinositol 3-kinase (PI3K)
pathway changes, FGFR1 amplification, and discoidin domain receptor tyrosine kinase 2 (DDR2) mutations not encountered, or relatively rarely so, in adenocarcinoma.

In summary, the Cancer Genome Atlas Project report has identified a number of potential therapeutic targets in patients with squamous cell carcinoma that need to be investigated in clinical trials, among them fibroblast growth factor receptor 1 (FGFR1) amplifications, discoidin domain receptor 2 (DDR2) mutations, and P13K alterations. Molecular alterations described in squamous cell carcinoma tumors of the lung that may well be targetable are FGFR1 amplification; DDR2 mutation; mesenchymal-epithelial transition factor (MET) mutation/amplification; phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit a (PIK3CA) mutation/amplification; PTEN loss; SOX2 amplification; AKT1 mutation; and TP53 mutation. These are discussed individually here.

**FGFR1**

The FGFR signaling pathway normally contributes to the physiological processes of tissue repair, hematopoiesis, angiogenesis, and embryonic development.11 There are 4 members in the FGFR family, and 22 known fibroblast growth factor (FGF) ligands. Activation of this pathway leads to enhanced growth of NSCLC cells. Furthermore, FGFR1 amplification has been identified as a potential relevant target in squamous cell carcinoma of the lung. Weiss et al reported that 22% of 153 samples of squamous cell carcinoma tissue were found to have FGFR1 amplification by fluorescence in situ hybridization (FISH) analysis.12 Dutt et al identified FGFR1 amplification in 21% of 57 squamous cell carcinoma samples and in 3% of adenocarcinomas of the lung by single-nucleotide polymorphism array analysis.13 Both studies showed that cell growth in models harboring FGFR1 amplification depends on FGFR1. The percentage of stage IV squamous cell carcinoma patients with FGFR1 amplification tends to be around 20%.14 In a recent systematic review including 13 eligible studies analyzing FGFR1 in a total of 1798 squamous cell carcinoma patients, 19% of them were found to have FGFR1 amplification.15 Pearson’s correlation analysis suggested that smoking status was highly correlated with FGFR1 amplification.

Targeting the FGF/FGFR signaling pathway is among the strategies being explored in squamous NSCLC. That strategy is supported by the growth-promoting effects of the FGF signaling pathway observed in preclinical studies, as well as the finding that FGF/FGFR-related aberrations may be more common in squamous versus adenocarcinoma and other histological types.16

A number of FGFR inhibitors are now being analyzed in clinical trials in NSCLC patients (Table I). AZD4547, an FGFR1 inhibitor, is under clinical investigation in recurrent squamous cell carcinoma of the lung, both as a monotherapy and in combination with docetaxel in a phase 1/2 trial. The results of the phase 1 expansion of AZD4547 in patients with previously treated stage IV FGFR1-amplified squamous cell carcinoma of the lung were presented at the 2014 meeting of the American Society of Clinical Oncology (ASCO).17 Of 15 treated patients, 7 had FISH ratios ≥ 2.8 (high amplification), and a total of 14 patients were evaluable for tumor response: 1 with partial response, 4 with stable disease, and 9 with disease progression. The partial response was observed in a patient with high FGFR1 amplification. The authors concluded that AZD4547 has modest overall activity in patients with FGFR1-amplified stage IV squamous cell carcinoma of the lung, but did not meet the primary efficacy end point required for study continuation. BGJ398 is a pan-FGFR inhibitor being investigated in a dose-escalation trial for FGFR-amplified malignancies. The results with BGJ398 in a subgroup of squamous cell carcinoma patients with FGFR1 amplification treated in a phase 1 study were also presented at ASCO 2014.18 Of 26 evaluable patients, 4 (15.4%) achieved lasting partial response and 9 (34.6%) had stable disease. The results showing promising activity in squamous cell carcinoma patients with FGFR1 amplification have encouraged further development of BGJ398 in FGFR1-amplified squamous cell carcinoma patients. Dovitinib (TK258) is an inhibitor of FGFR3, and potentially other FGFRs, which is currently being tested in patients with advanced NSCLC or colorectal cancer and in patients with squamous cell carcinoma tumors of the lung. Nintedanib is being studied in phase 2 studies in patients with FGFR1-amplified NSCLC. BAY1163877, a pan-FGFR inhibitor, is being analyzed in a study in patients with FGFR-amplified cancers. GSK3052230, an antagonist of FGFR receptors, is a fusion protein composed of the extracellular domain of the American Society of Clinical Oncology (ASCO).17 Of 15 treated patients, 7 had FISH ratios ≥ 2.8 (high amplification), and a total of 14 patients were evaluable for tumor response: 1 with partial response, 4 with stable disease, and 9 with disease progression. The partial response was observed in a patient with high FGFR1 amplification. The authors concluded that AZD4547 has modest overall activity in patients with FGFR1-amplified stage IV squamous cell carcinoma of the lung, but did not meet the primary efficacy end point required for study continuation. BGJ398 is a pan-FGFR inhibitor being investigated in a dose-escalation trial for FGFR-amplified malignancies. The results with BGJ398 in a subgroup of squamous cell carcinoma patients with FGFR1 amplification treated in a phase 1 study were also presented at ASCO 2014.18 Of 26 evaluable patients, 4 (15.4%) achieved lasting partial response and 9 (34.6%) had stable disease. The results showing promising activity in squamous cell carcinoma patients with FGFR1 amplification have encouraged further development of BGJ398 in FGFR1-amplified squamous cell carcinoma patients. Dovitinib (TK258) is an inhibitor of FGFR3, and potentially other FGFRs, which is currently being tested in patients with advanced NSCLC or colorectal cancer and in patients with squamous cell carcinoma tumors of the lung. Nintedanib is being studied in phase 2 studies in patients with FGFR1-amplified NSCLC. BAY1163877, a pan-FGFR inhibitor, is being analyzed in a study in patients with FGFR-amplified cancers. GSK3052230, an antagonist of FGFR receptors, is a fusion protein composed of the extracellular domain of
**FGFR1** and the crystallizable fragment (Fc) of immunoglobulin G1 (IgG1). It is currently being examined in a phase 1 study both as a monotherapy and in combination with chemotherapy in squamous cell carcinoma patients with alterations in FGF receptors.

**DDR2**

DDR2 is a tyrosine kinase receptor that plays a role in cell adhesion, proliferation, and extracellular remodeling after binding of collagen, an endogenous ligand. In a study by Ford et al., it was reported that upregulation of DDR2 was related to prolonged disease-free and overall survival of patients with NSCLC, especially those with squamous cell carcinoma.19

Hammerman et al examined 290 squamous cell carcinoma tissue samples and found the frequency of **DDR2** mutations to be 3.8%.20 **DDR2** mutations are driving molecular alterations, whose activation was inhibited by treatment with dasatinib, a multikinase inhibitor. They also presented details of a squamous cell lung cancer patient harboring a **DDR2** kinase domain mutation who responded to dasatinib and erlotinib treatment. A phase 2 trial is currently examining dasatinib in NSCLC patients with **DDR2** mutation.

**MET**

**MET**, also known as hepatocyte growth factor receptor (HGFR), is a proto-oncogene with relevant implications in NSCLC.21 **MET** mutations have been observed in about 3% of treatment-naive NSCLC patients, mainly within exons 2 and 14, outside of the kinase domain.22 Mutations in **MET** are observed more often in smokers. The **MET** mutation N375S was detected as a germline mutation in a high proportion of East Asian tissue samples, and was correlated to the incidence of squamous cell carcinoma.23

**MET** amplification occurs in 2% to 4% of NSCLC patients.24 Such amplifications occur at equal frequencies in squamous and adenocarcinomas with or without EGFR or Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations.25 Lung cancer cells with **MET** amplification are highly sensitive to **MET** inhibitors. A recent case report showed a major partial response to crizotinib (a dual **MET/ALK** inhibitor) in a patient with stage IV squamous cell carcinoma of the lung with **MET** amplification determined by FISH.26

**PI3KCA amplification/mutation**

**PI3KCA**, located on chromosome 3q26, encodes a class I PI3K catalytic subunit (p110α). The PI3K-**AKT** pathway plays a critical role in survival and growth of diverse cancer cells. Both copy number gain and mutations of **PI3K** are found in lung cancer. The copy number gain of **PI3K** is found in 33.1% of squamous cell carcinomas, in 6.2% of adenocarcinomas, and in 4.7% of small-cell lung cancers.27 Mutations of **PI3K** are described in 16% of squamous cell carcinoma of the lung.20,13,28

In a recent manuscript, the authors analyzed **PIK3CA** mutation in tumor tissue from 1144 NSCLC patients and identified 42 (3.7%) patients with mutations in exons 9 and 20.29 These mutations were found more often in squamous cell carcinoma (8.9%) than in adenocarcinoma (2.9%; \( P<0.001 \)) histological types. The most common **PIK3CA** mutation was exon 9 E545K. The majority of patients (57%) had additional oncogenic aberrations.

Inhibitors of this pathway are in development and clinical trials using a number of PI3K inhibitors are ongoing in patients with squamous cell carcinoma of the lung.

**PTEN loss**

**PTEN**, a tumor suppressor gene located on chromosome 10q23, encodes a lipid phosphatase inhibiting the PI3K-AKT pathway. Loss of **PTEN** activates PI3K-AKT signaling. Inactivation of **PTEN** by somatic **PTEN** deletions, mutations, and epigenetic mechanisms is found in many cancers. Reduction or loss of **PTEN** expression has been reported in up to 70% of NSCLC patients, in both adenocarcinoma and squamous cell carcinoma histological types.30 **PTEN** mutations, occurring in approximately 5% of lung cancers, are significantly associated with squamous cell rather than adenocarcinoma histological types.31 Patients with lung cancer showing **PTEN** loss may be more sensitive to inhibitors of the PI3K pathway; clinical trials of PI3K inhibitors for lung cancer patients with **PTEN**-loss tumors are ongoing.

**SOX2 amplification**

Amplification within chromosome 3q26 is a common genetic alteration found in squamous cell carcinoma of the lung. **SOX2** is a candidate oncogene present in this locus and amplification of **SOX2** has been reported in about 20% of squamous cell carcinoma cases.32 **SOX2** is a transcriptional factor that plays an important role in the regulation of stem cell function and the development of lung epithelium. Bass et al showed that inhibition of **SOX2** suppressed cell growth.33 Nevertheless, subsequent studies have confirmed that **SOX2** amplification is not enough for carcinogenesis to occur and additional mutations of downstream effectors are needed to develop a cancer.

**AKT1 mutation**

E17K somatic mutation of **AKT1**, located on chromosome 14q32, activates the protein kinase continuously. This mutation is known to be present in about 1% of squamous cell carcinomas and appears to be nonoverlapping with other driver mutations.34 **AKT** inhibitors are currently being studied in a number of trials. At present, the role of **AKT1** mutations in the selection of therapy is yet to be established.

**TP53 mutation**

**TP53**, a tumor suppressor gene, is located on chromosome 17p13 and encodes a protein functioning mainly as a tran-
The mutations are affected by smoking. In a substantial number of tumors, wild-type p53 is inactivated by overexpression or amplification of MDM2 proto-oncogene, E3 ubiquitin protein ligase (MDM2), which ubiquinates p53 and marks it for degradation. Currently, the presence or absence of TP53 mutation has no impact on the selection of therapy.

**Summary**

Recent randomized trials have produced positive findings in regard to new treatment options for patients with stage IV squamous cell carcinoma in both the first-line and second-line settings, including necitumumab and ramucirumab, among others. These compounds, together with anti–PD-1 and anti–PD-L1 strategies, will probably change the treatment algorithm in stage IV squamous cell carcinoma of the lung. In addition, recent studies have identified several potential therapeutic targets including FGFR1 amplifications, DDR2 mutations, and PI3K pathway alterations. Clinical trials with inhibitors of these pathways are ongoing, FGFR inhibitors being, at present, at the most developed stage.

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**References**

14. Weiss J, Sos ML, Seidel D, et al. Frequent and focal FGFR1 amplification asso-
Le cancer du poumon est la cause principale de décès par cancer dans le monde entier. Le cancer du poumon non à petites cellules compte pour environ 80 à 85 % des cancers du poumon et peut être divisé en deux types prédominants, l’adénocarcinome (environ 50 % des cas) et le carcinome épidermoïde (environ 30 % des cas). Tous les types histologiques de carcinome pulmonaire sont associés au tabagisme, l’association étant plus forte pour les carcinomes épidermoïdes et à petites cellules. Les mutations du récepteur du facteur de croissance épidermique (EGFR) ou les fusions du récepteur de la tyrosine kinase (ALK) des lymphomes anaplasiques au cours des carcinomes épidermoïdes sont rares. Les traitements ciblés efficaces contre les adénocarcinomes sont inefficaces ou contre-indiqués dans les carcinomes épidermoïdes, pour lesquels les options thérapeutiques sont limitées. Récemment, le profilage moléculaire des carcinomes épidermoïdes du poumon a montré des transformations pouvant être ciblées : parmi celles-ci, des amplifications du récepteur du facteur de croissance du fibroblaste (FGFR), des mutations du récepteur de la tyrosine kinase 2 à domaine discoidine (DDR2) et des mutations/amplifications de la sous-unité catalytique, phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3CA). Actuellement, un grand nombre d’études cliniques utilisant des inhibiteurs du FGFR sont en cours chez des patients atteints de carcinomes épidermoïdes. De plus, l’exploration des points de contrôle immunitaires a permis de nouvelles immunothérapies conçues pour interrompre la signalisation par l’intermédiaire de la voie de la protéine 1 de mort cellulaire programmée dans les lymphocytes. La modulation de cette voie peut restaurer les réponses immunitaires antitumorales et les données préliminaires montrent clairement son activité dans le carcinome épidermoïde du poumon. Cet article présente brièvement le traitement standard maintenant disponible pour les patients ayant un carcinome épidermoïde de stade IV ainsi que l’expérience actuelle des modifications moléculaires dans ce sous-groupe, en se concentrant en particulier sur les modifications susceptibles d’être ciblées thérapeutiquement.
Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphomas. Recently, molecular studies have revealed some DLBCL subtypes that have distinct clinical courses, responses to treatment, and prognoses. DLBCL is associated with an aggressive natural medical history, but the combination of chemotherapy—e.g., CHOP regimen (cyclophosphamide, hydroxydaunorubicin, vincristine [Oncovin], and prednisone)—with anti-CD20 monoclonal antibody therapy has greatly improved the outcome of the disease and has enabled approximately 70% of patients to be cured. However, substantial progress is still required, particularly for relapsed/refractory disease and in the frontline treatment of elderly and frail patients. Future approaches to DLBCL treatment will rely on new cytotoxic drugs with a high efficacy/toxicity ratio and therapies directed against the targets identified by the new genetic subclassification of DLBCL.

Medicographia. 2015;37:263-270 (see French abstract on page 270)
DLBCLs: treatment paradigms and unmet medical needs – Solal-Celigny

**SELECTED ABBREVIATIONS AND ACRONYMS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>aalPI</td>
<td>age-adapted International Prognostic Index</td>
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<td>ABC</td>
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<td>ASCT</td>
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<tr>
<td>R-EPOCH</td>
<td>rituximab plus etoposide, prednisone, vincristine (Oncovin), cyclophosphamide, and hydroxydaunorubicin (doxorubicin)</td>
</tr>
<tr>
<td>R-ICE</td>
<td>rituximab plus ifosfamide, etoposide, and carboplatin</td>
</tr>
<tr>
<td>R-IPI</td>
<td>revised International Prognostic Index</td>
</tr>
<tr>
<td>R/R</td>
<td>relapsed/refractory</td>
</tr>
</tbody>
</table>

...ontation and at relapse; age >60 years, performance status >1, Ann Arbor stage III-IV, number of extranodal involvement site(s) >1, and serum lactate dehydrogenase (LDH) level above normal, with 1 point given for each factor. Four risk groups have been identified: low (0-1 factor), low-intermediate (2 factors), high-intermediate (3 factors) and high risk (>3 factors), with 5-year overall survival (OS) ranging from 73% to 26% in the initial report carried out on patients treated with anthra- cycline-based chemotherapy only. A simplified score, the de- nominated age-adapted IPI (aalPI), has been proposed for patients who are less than 60 years of age and relies on 3 pa- rameters: an above-normal serum LDH level, Ann Arbor stage III-IV, and ECOG (Eastern Cooperative Oncology Group) per- formance status >1. The aalPI stratifies patients into 4 discrete groups utilizing these parameters to elicit a score from 0 to 3 for adverse factors. It has also been validated in patients >60 years. Although constructed and validated in the pre-rituximab era, the validity of the IPI was also confirmed in patients treated with rituximab plus chemotherapy. For patients treated with a combination of chemotherapy and rituximab, a re- viscied IPI (R-IPI) was proposed by redistributing the IPI factors into 3 prognostic subgroups: “very good” group (0 factors), “good” (1 or 2 factors) and “poor” (3 factors). The range of outcomes has narrowed substantially and all risk groups have at least a 50% chance of being cured. However, the IPI, whether in its initial or revised form, has only a limited ability to identify a subgroup with substantially poor outcome. Cur- rently, the original IPI/aalPI and the R-IPI remain the prospec- tively designed and validated measures for assessing DLB- CL risk.

However, clinical indices are surrogate markers for underlying biological differences between patients and a large number of biological tools are now proposed to evaluate the risk and predict the outcome for patients.

**Molecular and genetic prognostic factors**

Gene-expression profiling (GEP) identifies 3 distinct forms of DLBCL: the germinal center B-cell (GCB), activated B-cell (ABC), and primary mediastinal B-cell subtypes. Approximately 15% of cases do not fit into any of these categories. Each of these categories have distinct counterparts in the normal maturation/activation B-cell development cycle. GCBs arise from centrogerminative centroblasts. ABCs arise from proplasmablastic B-cells and have always had a poorer prognosis than DLBCL with a GCB signature, even in the rituximab era. The primary mediastinal B-cell subtype arises from a rare and specific thymic B cell and has genetic commonalities with the cell of origin of classical Hodgkin lymphoma. As GEP techniques are costly and cumbersome, surrogate immuno- histochemistry-based algorithms have been proposed. The Hans algorithm, based on the presence of 3 markers (CD10, B-cell chronic lymphocytic leukemia/lymphoma 6 (BCL6) and melanoma associated antigen (mutated) 1 (MUM1)), is the most widely used, although concordance with...
GEP does not exceed 80%. These molecular subtypes are associated with distinct oncogenic pathways that can be exploited differentially by new, targeted therapies. These include the GC-DLBCL–expressed genes normally detected in GCB cells such as CD10, LIM domain only 2 (LMO2), and the transcriptional repressor BCL6. The pathogenetic hallmark of ABC-DLBCL is the constitutive activation of the nuclear factor xB (NF-xB) signaling pathway.

GEP studies have also identified molecular signatures related to the microenvironment that correlated with outcome independently of lymphoma-cell GEP profile. The stromal-1 signature reflects extracellular matrix deposition and tumor infiltration by macrophages and is associated with a favorable outcome. The stromal-2 signature identifies tumors with a high level of angiogenesis and a high density of blood vessels and is associated with a poorer outcome.9

B-cell chronic lymphocytic leukemia/lymphoma 2 (BCL2) is an antiapoptotic protein that plays a major role in normal B-cell development. BCL2 overexpression has been reported in approximately 50% of DLBCL cases. Overexpression of BCL2 has a negative influence on clinical outcome for the GCB subtype, but not for the ABC subtype.

MYC protein overexpression—which is a constitutive hallmark of Burkitt lymphoma and related to MYC oncogene rearrangement—has been found in 5%-10% of DLBCLs and is associated with a poor outcome. DLBCL cases that overexpress both BCL2 and MYC proteins represent 5% of DLBCL cases and have always had a dramatically poorer outcome than the others, independently of the IPI and of the cell of origin, even in the rituximab era.9

**Response to treatment**

Complete remission (CR) after treatment is mandatory, but not sufficient, to reach the goal of cure in DLBCL patients. Consensual criteria for defining response to treatment, often reported as the Cheson criteria, were established in 2007, revised in 2014, and have to be used in routine practice and in clinical trials.10,11 CR rates are much lower after salvage treatment for progression/relapse than after initial treatment.

Among the criteria used for assessing response to treatment, positron emission tomography (PET)-computed tomography (CT) with 18fluorodeoxyglucose (FDG) has become the imaging standard. At presentation, DLBCL cases have a high uptake rate for FDG and PET-CT is the optimal method for evaluating the extent of the disease. At the end of therapy, measuring FDG uptake is the most accurate method to evaluate treatment efficacy. Results of final PET-CT can be evaluated either by visual assessment or using a semiquantitative method based on standardized uptake value (SUV). A positive PET-CT scan at the end of treatment is highly predictive of residual or recurrent disease and is associated with an inferior progression-free survival (PFS) and OS.12 Interim PET-CT after 2 cycles of chemotherapy in DLBCL patients is used by many clinicians in order to assess efficacy as soon as possible, but results are conflicting and should not influence treatment outside a clinical trial.

**Frontline treatment**

If left untreated, DLBCL has a median survival of less than 1 year. For the last 40 years, anthracycline-based chemotherapy regimens formed the basis of treatment. The introduction of the chimeric monoclonal anti-CD20 antibody 15 years ago was a milestone in the treatment of DLBCL, greatly improving both PFS and OS. This improvement in outcome for patients with DLBCL treated with a modern approach is illustrated by the observational study of 1366 patients treated in British Columbia in the rituximab era (2001-2011), which shows an OS of 60% at 10 years. Since very few relapses occur after this time lag, these patients can be considered cured.13 The paradigms of the most widely used treatments in adult patients are illustrated in Figure 1 and will be further detailed.14 The main chemotherapy regimens used in DLBCL patients are shown in Table I (page 266).

**Treatment of “young” patients (aged 18-65 years)**

The treatment is stratified according to disease risk assessed by the IPI score. In patients with no more than 1 risk factor, 6 cycles of R-CHOP (rituximab plus cyclophosphamide, hydroxydaunorubicin [also called doxorubicin or Adriamycin], vincristine [Oncovin], and prednisone) are administered at 21-day intervals; RT, radiotherapy.
Oncovin [vincristine], and prednisone) is the mainstay of most treatment regimens. R-CHOP21 (R-CHOP, at 21-day intervals) is the most widely used treatment, but in many centers, R-CHOP14 (R-CHOP, at 14-day intervals) is used, which allows a shorter treatment duration. However, the toxicity, particularly the myeloid toxicity, of R-CHOP14 is superior to that of R-CHOP21. Two randomized studies have compared these two regimens and reached the same conclusion; namely that R-CHOP14 was not superior to R-CHOP21 in terms of response rate and PFS, despite a higher dose-intensity of chemotherapy. There is much controversy over the benefits of consolidation involved-field radiotherapy in patients with low-risk IPI (0-1) and localized disease. Radiotherapy may be proposed to patients with initial site(s) of bulky disease (ie, >5 cm) and to site(s) which remain PET-positive at the end of treatment. In the MInT trial (MabThera International Trial), the 6-year PFS and OS of 824 patients with 0 or 1 risk factors were respectively 80% and 89.8%.

Treatment of “young” patients with high-intermediate–risk or high-risk IPI score with R-CHOP yields less satisfactory results with a 3-year PFS that does not exceed 50%. Several options have been tested in order to improve these results, but none of them has clearly demonstrated its superiority and a consensus is difficult to reach. Schematically, 3 treatment methods have been tested separately or in combination:

- Increasing dose
- Increasing the number of drugs
- Including high-dose therapy with autologous stem cell transplantation

Several regimens that increase doses of cytotoxic drugs have been designed. The LNH03-2B conducted by GELA (Groupe d’Etudes des Lymphomes de l’Adulte) compared 8 cycles of R-CHOP21 with a R-ACVBP regimen (rituximab plus Adriamycin [doxorubicin], cyclophosphamide, vindesine, bleomycin, and prednisone; see Table I), which is much more intense than R-CHOP, in patients with 1 risk factor according to aIPI. Patients treated with R-ACVBP had a better 3-year event-free survival (EFS) and OS than those treated with R-CHOP (respectively 81% vs 67% and 92% vs 84%). However, the severe toxicities observed in patients treated with ACVBP limited its use. Other dose-dense regimens such as the R-MegaCHOP (R-CHOP regimen, increasing doses from cycle to cycle) have also been proposed.

### Table I. Main chemotherapy regimens used in diffuse large B-cell lymphoma.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Drugs</th>
<th>Delay between cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-CHOP21</td>
<td>Rituximab 375 mg/m² D1, Doxorubicin 50 mg/m² D1, Cyclophosphamide 750 mg/m² D1, Vincristine 1.4 mg/m² D1, Prednisone 40 mg/m² D1-D5</td>
<td>21 days</td>
</tr>
<tr>
<td>R-CHOP14</td>
<td>Rituximab 375 mg/m² D1, Doxorubicin 50 mg/m² D1, Cyclophosphamide 750 mg/m² D1, Vincristine 1.4 mg/m² D1, Prednisone 40 mg/m² D1-D5</td>
<td>14 days</td>
</tr>
<tr>
<td>R-ACVBP</td>
<td>Rituximab 375 mg/m² D1, Doxorubicin 75 mg/m² D1, Vindesine 2 mg/m² D1-D5, Bleomycin 10 mg D1-D5, Cyclophosphamide 1200 mg/m² D1, Prednisone 60 mg/m² D1-D5</td>
<td>14 days</td>
</tr>
<tr>
<td>BEAM</td>
<td>Carmustine 300 mg/m² D1, Etoposide 200 mg/m² D1-D2, Cytarabine 200 mg/m² D1-D2, Melphalan 140 mg/m² D1, Stem cell transplantation D0</td>
<td></td>
</tr>
<tr>
<td>R-miniCHOP</td>
<td>Rituximab 375 mg/m² D1, Doxorubicin 25 mg/m² D1, Cyclophosphamide 400 mg/m² D1, Vincristine 1 mg/m² D1, Methyprednisolone 40 mg/m² D1</td>
<td>21 days</td>
</tr>
<tr>
<td>R-DHAP</td>
<td>Rituximab 375 mg/m² D1, Dexamethasone 40 mg/m² D1-D4, Cytarabine 2 g/m² D1, Cisplatina 100 mg/m² D1</td>
<td>21 days</td>
</tr>
<tr>
<td>R-ICE</td>
<td>Rituximab 375 mg/m² D1, Carboplatin AUC 5 D2, Etoposide 100 mg/m² D1-D3, Ifosfamide 5 mg/m² D2</td>
<td></td>
</tr>
</tbody>
</table>

* May be replaced by oxaliplatin 130 mg/m² or carboplatin AUC 5.
chemotherapy followed by HDT and ASCT. In all of these trials, no improvement in OS in patients treated with HDT and ASCT was observed. HDT and ASCT as frontline treatment remains a potentially useful option in “young” patients with high-risk DLBCL, but is not recommended to be used outside a clinical trial.

In contrast to follicular and mantle cell lymphomas, no available maintenance treatment demonstrated efficacy in DLBCL.

The issue of prophylaxis of central nervous system (CNS) relapse remains a subject of debate. Some patients are at higher risk of CNS relapse: patients with testicular, bone marrow, or head-and-neck involvement, more than 1 extranodal involvement site, or who are in a high-risk IPI group. These patients usually receive 4 intrathecal methotrexate injections for prophylaxis although there is no demonstration of the efficacy of this treatment. Intrathecal methotrexate does not prevent parenchymal CNS relapse. Some data suggest that systemic high-dose methotrexate may be more effective in the prevention of all types of CNS relapse.

Treatment of patients aged over 65 years
Figure 2 shows a proposed treatment scheme for DLBCL patients who are over 65 years of age. Age is a major adverse prognostic factor in DLBCL. This effect is related to:

* Intrinsic factors of DLBCL in elderly patients, such as a greater proportion of non-GC DLBCL and a high frequency of Epstein-Barr virus (EBV)-related DLBCL, which has a poorer outcome than other de novo DLBCL.
* Age-related decline in renal and liver functions, with well-established consequences on drug metabolism and toxicity.
* Decrease in marrow reserve and increased risk of myelosuppression.
* Comorbidities whose incidence increases with age, especially in patients aged 75 years or older.
* Cardiovascular disease, often clinically asymptomatic, which increases the risk of anthracycline-related cardiomyopathy.

All these reasons justify a specific evaluation of DLBCL patients aged 70 years or older. The Comprehensive Geriatric Assessment (CGA) is an exhaustive evaluation of the medical, cognitive, socioeconomic, and psychological status of elderly patients, which provides an optimal proposal for a treatment plan and enables a supportive care program to be established in order to improve treatment tolerance. However, CGA takes up to 2 hours to be carried out and a far more simplified, score-dominated Geriatric 8 (G8) screening tool has recently been proposed that provides accurate detection of frail patients potentially unfit for optimal treatment and for whom a complete CGA assessment may be performed before choosing a treatment.

An optimal frontline treatment regimen for DLBCL patients includes full-dose doxorubicin (Adriamycin), providing that the pretreatment evaluation suggests that it can be tolerated. This requires measurement of the left ventricular ejection fraction (LVEF) either by echocardiography or by multiple-gated acquisitions scanning. Only patients with an LVEF of at least 50%-55% may be able to tolerate full-dose doxorubicin (Adriamycin). Whenever feasible, R-CHOP21 is the best initial treatment, even in elderly patients. An initial prephase treatment with prednisone and low-dose vincristine may improve the performance status of elderly patients and enable full-dose R-CHOP immunochemotherapy to be subsequently administered.

Several regimens have been tested in patients too frail to receive full-dose R-CHOP. In a study conducted by the LySA group (Lymphoma Study Association), carried out on 150 patients aged over 80 years, an R-CHOP regimen with decreased doses of doxorubicin (Adriamycin) and cyclophosphamide (R-miniCHOP) yielded a response rate of 73% and a median PFS of 21 months. Other cytotoxic drugs with low toxicity still need to be tested in this setting. Epirubicin and idarubicin have been tested and were less cardiotoxic than doxorubicin (Adriamycin) at doses used in clinical trials, but their equiva-
Treatment of relapsed/refractory DLBCL

Despite overall improvements in the outcome of DLBCL, approximately 40% of patients develop relapsed/refractory (R/R) disease. Biopsy will be required in most cases of suspected R/R disease given the prognostic and therapeutic impact and the fact that some other disorders (sarcoidosis, carcinoma) may mimic R/R disease. After confirmation, patients should undergo restaging procedures.

R/R disease may be separated into 4 subgroups: (i) refractory with disease progression during treatment, which is associated with an extremely poor prognosis; (ii) partial response after treatment; (iii) early relapse, ie, relapse within 6-12 months after the end of previous treatment; (iv) late relapse with, in some cases, a more indolent histological type. In addition to these categories, the IPI at relapse has to be used to assess prognosis. It must be stated that the results of salvage treatments are poorer in patients previously treated with rituximab-based immunochemotherapy in comparison with patients who are rituximab-naive. At the present time, almost all patients treated for DLBCL fall in this category.

Firstly, it must be established whether or not the patient is eligible for HDT and ASCT. Age >70 years is a widely used threshold to rule out such a treatment. In patients aged less than 70 years, severe cardiac, pulmonary and/or liver comorbidities are contraindications to HDT. The PARMA trial (trial name not an acronym) conducted more than 20 years ago has clearly demonstrated the benefits of HDT and ASCT for patients whose relapse remains chemosensitive.

For patients who are eligible for HDT, the first goal is to administer a non–cross-resistant salvage regimen. Although there is no clear demonstration that adding rituximab to salvage chemotherapy adds efficacy in patients previously treated with R-chemotherapy, most patients are treated with such a combination at the time of relapse. The most widely used chemotherapies contain a platinum derivative: DHAP (dexamethasone, high-dose Aracytine [cytarabine], and Platinol [cisplatin]), ICE (ifosfamide, carboplatin, and etoposide) (Table I). Three to four cycles are given and stem cells are collected after the second and/or third cycle.

A PET scan is the most accurate means of evaluating chemosensitivity. In the CORAL study (COllaborative trial in Relapsed Aggressive Lymphoma), patients with R/R DLBCL were randomly allocated to R-ICE (rituximab plus ICE) or R-DHAP (rituximab plus DHAP). Overall, there was no difference in outcome between these 2 regimens. With R-DHAP or R-ICE, the response rate was 63% and only 50% were able to proceed to ASCT.

There is no standard conditioning regimen for ASCT in patients who have responded to salvage therapy. A combination of carmustine (BCNU), etoposide, cytarabine (Aracytine), and melphalan is the most widely used treatment. Approximately 30% of patients who are able to receive the whole procedure do not relapse. Maintenance treatment with rituximab after ASCT did not decrease the relapse rate.

The outcome of patients who are not sensitive to salvage therapy or who relapse after HDT is very poor, with a median survival of less than 6 months. In patients less than 55-60 years of age, an allogeneic stem cell transplantation may be planned. For other patients, there is no demonstration that aggressive treatment improves survival. Less-intensive approaches or participation in a clinical trial are justified approaches.

The prognosis of patients who are not eligible for HDT and ASCT is also poor. After intensive salvage chemotherapy regimens such as R-DHAP or R-ICE, the relapse rate is higher than 80%. The toxicities of these regimens must be taken into account in this setting. Other regimens, such as combinations of gemcitabine and oxaliplatin have been tested, yielding a median PFS of less than 6 months. Monotherapy with pixantrone may be proposed to patients in second or subsequent relapse. Whenever possible, these patients should be referred for clinical trials of new drugs since treatment requirements have not been met within this context.

New therapies for DLBCL

The poor prognosis of DLBCL for patients who are not cured by frontline immunochemotherapy, as well as the increasing incidence of the disease in elderly patients who cannot be treated with conventional treatment, underline the fact that new approaches are clearly needed. The improving understanding of DLBCL subtypes with their specific signaling pathway disturbances has led to the development of new targeted drugs.

Lenalidomide is an immunomodulatory agent that has numerous other mechanisms of action. It acts on the microenvironment, enhancing the cytotoxic activity of T and natural killer (NK) cells and decreases proliferation and angiogenesis. A clinical activity in heavily pretreated patients has been demonstrated in phase 1 and 2 trials. In a large phase 2 trial being carried out on 217 heavily pretreated patients, combinations of lenalidomide with R-CHOP or lenalidomide maintenance after R-CHOP in high-risk patients are currently being tested.

New anti-CD20 monoclonal antibodies (ofatumumab, obinutuzumab), which potentially have greater efficacy than rituximab, are under development in combination with CHOP chemotherapy. Antibody-drug conjugates combine a monoclonal antibody directed against a B-cell antigen closely linked to a cytotoxic agent. The antibody enables B cells to be tar-
targeted specifically and, after internalization of the complex, the cytotoxic drug acts on DNA and/or microtubules. Inotuzumab ozogamicin is an antibody against CD22 conjugated with calicheamicin, a potent inhibitor of microtubule organization.

Enzastaurin is a protein-kinase Cβ inhibitor. GEP studies showed an overexpression of this kinase in relapsed DLBCL and early phase trials suggested efficacy. Unfortunately, a clinical trial of enzastaurin as a maintenance treatment in high-risk DLBCL patients who had responded to R-CHOP did not confirm efficacy in this setting.

The B-cell receptor (BCR) signaling pathway is critical to the development and maturation of normal B cells. Ibrutinib is a potent irreversible inhibitor of Bruton’s tyrosine kinase, a key kinase that plays a significant role in signal transduction in the BCR pathway. Ibrutinib has been tested in combination with R-CHOP in a phase 1b trial and has produced promising results. Bortezomib inhibits the NF-κB signaling pathway, which is constitutively activated in DLBCL of the ABC subtype. Preliminary studies have shown that bortezomib increases the response rate when combined with an R-CHOP regimen. A large phase 3 study is being conducted to evaluate the efficacy of adding bortezomib to standard immunotherapy in the R/R and frontline settings.

**Conclusion**

For over 40 years, no chemotherapy regimen has been produced having a better efficacy/toxicity than CHOP in the frontline treatment of DLBCL. In combination with rituximab, it enables approximately 70% of patients to be cured. However, unmet medical needs still exist for DLBCL, particularly in the frontline treatment of elderly and frail patients, and in the relapse setting for patients who are unfit for ASCT or who relapse afterwards. Improvements will be obtained from a better understanding of lymphomagenesis and better risk-stratification of patients taking into account molecular subtyping. A rational combination of new agents tested in well-designed clinical trials is needed.

**References**


**Keywords:** chemotherapy; CHOP, DHAP; diffuse large B-cell lymphoma; EPOCH, gene-expression profiling; ICE; monoclonal antibody therapy; relapsed/refractory

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**Lymphomes non hodgkiniens diffus à grandes cellules B : modèles de traitement et besoins médicaux non satisfaits**

Le lymphome diffus à grandes cellules B (LDGCB) est le sous-type de lymphome non Hodgkinien le plus courant. Récemment, il est apparu dans des études moléculaires que certains sous-types de LDGCB ont des évolutions cliniques, des réponses au traitement et des pronostics différents. L’évolution naturelle du LDGCB est agressive, mais l’association d’une chimiothérapie, par exemple le protocole CHOP (cyclophosphamide, hydroxydaunorubicine, vincristine [Oncovin] et prednisone) avec des anticorps monoclonaux anti-CD20, a grandement amélioré l’évolution de la maladie et a permis à environ 70 % des patients d’être guéris. Des progrès conséquents sont cependant encore nécessaires, en particulier pour les rechutes ou les cas réfractaires et pour le traitement de première ligne des patients âgés et fragiles. Les futurs traitements du LDGCB reposeraient sur les nouveaux médicaments cytotoxiques au rapport efficacité/ toxicité élevé et sur les traitements dirigés contre des cibles identifiées par la nouvelle sous-classification génétique du LDGCB.
Bispecific effector-cell engagers—novel immunotherapeutics trained to fight cancer by D. Baty, B. Kerfelec, and P. Chames, France

Bispecific effector-cell engagers are bispecific antibodies that simultaneously target a tumor-associated antigen and an activating receptor at the surface of effector cells, such as natural killer or T cells. In recent years, the development of antibody engineering has fostered the emergence of several new bispecific formats, leading to remarkable preclinical results in vitro and in vivo. Many of these formats are currently under intense clinical investigation. Furthermore, the end of 2014 saw for the first time in history the approval of a recombinant bispecific T-cell engager by the US Food and Drug Administration, namely blinatumomab (BLINCYTO™), for the treatment of relapsed or refractory B-cell precursor acute lymphoblastic leukemia. This review discusses the different formats designed to produce such immunotherapeutics with a special emphasis on molecules under clinical investigation. Several of these molecules are expected to dramatically improve treatment of a number of malignancies.

Antibody-based therapeutics are currently the fastest growing segment of the drug and biologics market. Since the launch of the first anti-CD3 antibody, muromonab-CD3 (OKT3), in 1986, close to 40 monoclonal antibodies (mAbs) and derivatives have been approved in the United States and Europe, and many of them are dedicated to cancer therapy. The first generation of approved antibodies were naked chimeric, humanized, or human antibodies endowed with several modes of action, mainly including the blockade of signaling pathways with induction of apoptosis, recruitment of the complement system (complement-dependent cytotoxicity [CDC]), or recruitment of effector cells such as natural killer (NK) cells (antibody-dependent cell-mediated cytotoxicity [ADCC]) or macrophages (antibody-dependent cell-mediated phagocytosis) through binding of the activating receptor FcγRIIA (or CD16A).1

Because ADCC is thought to be an important mode of action of several of these approved molecules, many efforts have been made to improve the interaction between the crystalizable fragment (Fc) of these molecules and FcγRIIA, through Fc or glycoengineering. This led to the approval of obinutuzumab, an anti-CD20 mAb that clearly outperforms rituximab, the first-generation anti-CD20 mAb. While many other ADCC-enhanced antibodies are in clinical trials, 2 new families of mAb-based therapeutics—antibody-drug conjugates (ADCs) and bispecific antibodies (bsAbs)—have emerged and led to exciting preclinical and clinical results. The high therapeu-
tic potential of ADCs is out of the scope of this review and has been thoroughly described in recent publications. The second family corresponds to bsAbs. The recent blossoming of this field of research is in contrast with its long history, which began years ago, soon after scientists could produce mAbs in a reliable fashion. This is explained by immunogenicity issues faced by murine antibodies, by the very poor production yields afforded by initial approaches used to build these molecules, such as chemical cross-linking of 2 different antibodies or using quadroma technology, and by the difficulty of combining both favorable pharmacokinetic properties and easy large-scale manufacturing into an unique new bispecific format.

The development of antibody engineering has brought about innovative solutions that have profoundly changed the situation. Many laboratories are currently exploring the numerous possibilities offered by bsAbs. A first therapeutic approach based on these molecules is the simultaneous blockage of 2 receptors or 2 ligands, allowing the simultaneous inhibition of redundant signaling pathways. The second approach, which constitutes the main focus of this review, is the recruitment and the activation of immune effector cells in the tumor microenvironment (Figure 1). The main actors of an immune response against tumor cells are NK cells, macrophages, and T cells. As a consequence, the large majority of bsAbs in this class target CD16 (FcyRIII) expressed by NK and macrophages, or CD3, expressed by T cells. Here, we will review the main preclinical and clinical results obtained with the various proposed formats of anti-CD3 or anti-CD16 bsAbs (Table I).

bsAbs: a tale of formats

For 2 decades, low yield and heterogeneity of bsAb production relying on methods such as hybrid hybridomas and chemical linking have been significant obstacles to their development. A turning point was reached with the capability to produce recombinant fragments of antibodies that possess the full binding activity of the entire immunoglobulin G (IgG) molecule. The antigen-binding fragment (Fab) corresponds to the association of the entire light chain covalently linked via a disulfide bond to the variable (VH) and first constant domains (C_{H1}) of the heavy chain. A smaller fragment could also be produced by linking the variable domains of the heavy and light chains (V_{H}·V_{L}) via a flexible peptide linker, leading to the so-called single-chain variable fragment (scFv). The possibility to produce these fragments in E. coli and to combine them as building blocks to create multispecific molecules has led to a plethora of bspecific fragments that have been recently reviewed in detail. In this review, we will focus our attention on the formats used in clinical trials. Most bsAb formats can be categorized as either small bspecific formats or IgG-like molecules, the fundamental difference lying in the presence or absence of an intact Fc portion (Figure 2, page 274).

The main benefits of IgG-like formats include a long serum half-life, owing to neonatal Fc receptor (FcRn) binding via the Fc portion, and that their production and purification can be compatible with well-established processes developed for conventional antibodies. By contrast, small molecules devoid of Fc are characterized by a short, or even very short, serum half-life if their molecular weight is below the threshold of renal clearance (60-70 kDa). This drawback can be circumvented by continuous infusion protocols or partly solved by fusion with an albumin-binding domain. Moreover, the wide variety of size and valencies afforded by the modular nature of small formats and the frequent presence of linkers can also induce a lower global stability and, in some cases, aggregation issues, which in turn might increase their overall immunogenicity. On the other hand, the compact size might be an advantage when

### Selected abbreviations and acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADC</td>
<td>antibody-drug conjugate</td>
</tr>
<tr>
<td>ADCC</td>
<td>antibody-dependent cell-mediated cytotoxicity</td>
</tr>
<tr>
<td>ATC</td>
<td>activated T cells</td>
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<tr>
<td>Blf</td>
<td>bispecific immunofusion</td>
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<tr>
<td>BiTE</td>
<td>Bispecific T-cell Engager</td>
</tr>
<tr>
<td>bsAb</td>
<td>bispecific antibody</td>
</tr>
<tr>
<td>CEA</td>
<td>carcinoembryonic antigen</td>
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<tr>
<td>C_{H1}</td>
<td>first constant domain of the antibody heavy chain</td>
</tr>
<tr>
<td>CLL</td>
<td>chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>Ck</td>
<td>constant domain of the kappa light chain</td>
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<tr>
<td>DART</td>
<td>Dual-Affinity Re-Targeting</td>
</tr>
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<td>Db</td>
<td>diabody</td>
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<tr>
<td>DC</td>
<td>dendritic cell</td>
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<tr>
<td>EC_{50}</td>
<td>half-maximal effective concentration</td>
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<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
</tr>
<tr>
<td>EpCAM</td>
<td>epithelial cell adhesion molecule</td>
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<tr>
<td>ErBB2</td>
<td>receptor tyrosine-protein kinase ErbB2 (formerly HER2)</td>
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<tr>
<td>Fab</td>
<td>fragment, antigen-binding [of immunoglobulin]</td>
</tr>
<tr>
<td>Fc</td>
<td>fragment, crystallizable [of immunoglobulin]</td>
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<tr>
<td>FcγR</td>
<td>receptor for Fc fragment of immunoglobulin G</td>
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<tr>
<td>FDA</td>
<td>US Food and Drug Administration</td>
</tr>
<tr>
<td>Fv</td>
<td>fragment, variable [of immunoglobulin]</td>
</tr>
<tr>
<td>gpA33</td>
<td>glycoprotein A33 antigen</td>
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<td>IgG</td>
<td>immunoglobulin G</td>
</tr>
<tr>
<td>mAb</td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>NHL</td>
<td>non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>NK</td>
<td>natural killer</td>
</tr>
<tr>
<td>OKT3</td>
<td>muramonoab-CD3</td>
</tr>
<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PSMA</td>
<td>prostate-specific membrane antigen</td>
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<tr>
<td>scDb</td>
<td>single-chain diabody</td>
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<tr>
<td>scFv</td>
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<td>tandem diabody</td>
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<td>Tb35</td>
<td>anti-CD3 Tribody</td>
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<tr>
<td>TCR</td>
<td>T-cell receptor</td>
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The development of antibody engineering has brought about innovative solutions that have profoundly changed the situation. Many laboratories are currently exploring the numerous possibilities offered by bsAbs. A first therapeutic approach based on these molecules is the simultaneous blockage of 2 receptors or 2 ligands, allowing the simultaneous inhibition of redundant signaling pathways. The second approach, which constitutes the main focus of this review, is the recruitment and the activation of immune effector cells in the tumor microenvironment (Figure 1). The main actors of an immune response against tumor cells are NK cells, macrophages, and T cells. As a consequence, the large majority of bsAbs in this class target CD16 (FcyRIII) expressed by NK and macrophages, or CD3, expressed by T cells. Here, we will review the main preclinical and clinical results obtained with the various proposed formats of anti-CD3 or anti-CD16 bsAbs (Table I).
it comes to tumor penetration. It has also been suggested that small bsAb formats, by forcing close contacts between effector and target cells, trigger the formation of efficient immune synapses that could, in some cases, lead to the exclusion of some bulky receptors involved in negative costimulation. This fact might explain why formats such as Bispecific T-cell Engager (BiTE, see below) do not require costimulatory signals, such as CD28 engagement.

**Figure 1.** Recruitment of immune effector cells by bispecific antibodies for cancer therapy. Depending on the effector cells, the mode of action can be either antibody-dependent cell-mediated cytotoxicity, antibody-dependent cell-mediated phagocytosis, or cytotoxic T-cell reaction.

**Abbreviations:** ADCC, antibody-dependent cell-mediated cytotoxicity; ADCP, antibody-dependent cell-mediated phagocytosis; bsFab, bispecific Fab fragments; NK, natural killer; TAA, tumor-associated antigen; TCR, T-cell receptor.

### Bispecific antibodies in clinical trials

<table>
<thead>
<tr>
<th>Target 1</th>
<th>Target 2</th>
<th>Format (name)</th>
<th>Clinical trial ID (Phase)*</th>
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<tr>
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<td>CD3</td>
<td>TaFv (BITE, blinatumomab)</td>
<td>NCT02101853 (3)†</td>
<td>Amgen</td>
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### Approved bispecific antibodies

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<td>TaFv (BITE, blinatumomab)</td>
<td>3 December 2014 (FDA)</td>
<td>Amgen</td>
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* Other clinical trial identifiers and phase numbers.
† NCT00560794 (2); NCT00274742 (1); NCT01741732 (2); NCT01466179 (2); NCT01207368 (2); NCT01209286 (2); NCT01471782 (1 and 2); NCT02003222 (3); NCT02143414 (2); NCT02013167 (3); NCT02000427 (2).
‡ NCT0052457 (2).
§ NCT00526889 (2); NCT00836654 (2); NCT00352833 (2); NCT01815528 (2); NCT00326885 (2); NCT00563836 (2); NCT01065246 (2); NCT01248440 (2); NCT01504256 (2); NCT00377429 (2).
‖ NCT00521261 (1); NCT00244946 (1).
Small bispecific formats

Tandem scFv

The simplest form of small bsAbs is perhaps the covalent association of 2 scFvs via a third flexible linker, leading to the so-called tandem scFv format (TaFv). At the end of the 1990s, this format was chosen by the company Micromet Inc (now part of Amgen) to generate their Bispecific T-cell Engagers (BiTE). BiTEs are generated by fusing an anti-CD3 scFv to an anti-tumor associated antigen scFv via a short 5-residue peptide linker (GGGGS). In 1995, Kufer and colleagues produced such a tandem scFv, targeting epithelial cell adhesion molecule (EpCAM) and human CD3 in Chinese hamster ovary (CHO) cells. This new kind of bsAb proved to be highly cytotoxic at nanomolar concentrations against various tumor cell lines, using unstimulated human peripheral blood mononuclear cells (PBMCs) and in the absence of cosignaling. Later, Löffler et al published similar data obtained with a fusion between a murine anti-CD19 scFv and a murine anti-CD3 constant domain. Short and long linkers are indicated by black and gray lines, respectively.

Abbreviations: AD, anchoring domain; CH, constant domain of the heavy chain; CL, constant domain of the light chain; Fc, crystallizable fragment of IgG; IgG, immunoglobulin G; S-S, disulfide bond; SH, thiol group; VH, variable region of heavy chain; VL, variable region of light chain.
contrast with the majority of published studies based on anti-CD3 bispecific constructs. BiTEs have been demonstrated to induce immunological synapses identical to synapses induced by regular T-cell stimuli, even in the absence of major histocompatibility complex (MHC) class I molecules, as shown by the lysis of EpCAM-expressing K562 cells or -transfected rodent cells by human effector cells. The small size (60 kDa) of BiTEs, which ensures close proximity of T cells and target-cell membranes, might therefore be responsible for their high efficiency by leading to the active displacement of negative regulatory proteins from the forming synapse, as demonstrated in the case of CD45. In recent years, Micromet has developed a large BiTE platform, generating BiTEs against several tumor-associated targets such as EpCAM, receptor tyrosine-protein kinase ErbB2 (ERBB2, formerly HER2), carcinoma-associated antigen (CEA), Ephrin A2, CD33, and melanoma-associated chondroitin sulfate proteoglycan (MCSP). The most advanced molecule, the anti-CD19xCD3 blinatumomab, rapidly demonstrated impressive success in early clinical trials and, as a consequence, Micromet was acquired in 2012 by Amgen for $1.2 billion. More recently, the US Food and Drug Administration (FDA) granted blinatumomab breakthrough therapy designation for patients with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL). In a phase 2 multicenter single-arm open-label study for this indication, 42% of the 185 patients evaluated in the trial achieved complete remission or complete remission with partial hematologic recovery within 2 cycles of treatment. Among the responders, 75% even achieved a minimal residual disease response. Consequently, on December 2014, the FDA granted approval of blinatumomab, named BLINCYTO™, for the treatment of patients with Philadelphia chromosome-negative (Ph−) relapsed or refractory B-cell precursor ALL, making it the first bsAb approved by the FDA. Three other BiTEs, anti-EpCAMxCD3 (MT110), anti-CEAxCD3 (MT111, MEDI-565) and anti-prostate-specific membrane antigen (PSMA)xCD3 (MT112, BAY 2010112), as currently under phase 1 clinical investigation for multiple solid cancers (NCT00635596), gastrointestinal adenocarcinomas (NCT01284231), and prostate cancer (NCT01723475), respectively.

Of note, the company Immunocore has produced a fusion between an affinity-matured soluble T-cell receptor (TCR) targeting the HLA-A2/gp100 peptide-MHC complex and the N-terminus of an anti-CD3 scFv. This molecule named IMC-Gmp100, closely resembling a Fab-scFv bsAb, and active at picomolar-range concentrations in vitro, is under phase 2 clinical investigation for the treatment of late-stage melanoma (NCT01211262).

**Diabody and Dual-Affinity Re-Targeting**

Another effective format is the diabody (Db) format, originally developed by Holliger et al. Dbs are noncovalent dimers of scFv fragments from 2 different antibodies in which the reduced length of the peptide linker between $V_h$-$V_l$ of the same scFv fragment impedes intramolecular pairing, thus promoting/forcing the cross-pairing with the complementary domains of a second scFv fragment. These compact molecules are expressed at high yields in bacteria, and have been shown by structural experiments to adopt several conformations. This format has been further improved by the addition of an extra peptide linker between the 2 polypeptides in order to further decrease the amount of homodimers, yielding fragments called single-chain Dbs (scDb). Numerous studies have demonstrated the potency of these formats in preclinical studies. Examples of bsAb fragments with potential as therapeutic candidates include bispecific anti-CD19xCD3 and anti-CD19xCD16 Dbs which demonstrated a synergistic antitumor effect in a preclinical model of non-Hodgkin lymphoma (NHL), a promising anti-epidermal growth factor receptor (EGFR)xCD3 Db able to cure colon cancer-xenografted mice in combination with lymphokine-activated killer cells, and an effective anti-PSMAxCD3 Db for the treatment of prostate cancer-xenografted mice in the presence of peripheral blood lymphocytes. In 2010, Bonvini et al published a variation of the Db format targeting NK cells through CD16 and CD32B on B cells, by adding a free C-terminal cysteine on each chain of the Db, leading to an interchain disulfide bond upon chain association. This new format, named DART for Dual-Affinity Re-Targeting, showed extended storage and serum stability, combined with potent tumor cytolysis and autologous B-cell depletion in culture. In another study, they performed a side by side comparison of the DART and BiTE formats using the variable domains of blinatumomab. The CD19xCD3 DART molecules achieved an enhanced activity on all CD19-expressing target B cells evaluated using resting and prestimulated human PBMCs or purified effector–T-cell populations. Since then, the company MacroGenics has developed several DARTs. MGD006 (also encoded as S80880 by Servier, a partner of MacroGenics) targets the interleukin 3–receptor α chain (CD123), overexpressed on malignant cells in a wide range of hematological malignancies including acute myelocytic leukemia (AML) and myelodysplastic syndrome. MGD007 targets the glycoprotein A33 antigen (gpA33), a cell surface antigen expressed in more than 95% of primary and metastatic human colorectal cancers, including cancer stem cells. In preclinical studies, MGD007 mediated potent lysis of gpA33-positive colorectal cancer cells both in vivo and in vitro, and tumor growth inhibition was observed at very low doses. Recently, 2 phase 1 clinical trials were initiated with these 2 DARTs (NCT02152956 and NCT02248805 for MGD006 and MGD007, respectively).

**Tandem Dbs**

In a seminal work, Kiprianov et al proposed a new format based on the Db concept. They generated dimers of scDb (CD3xCD19) in an antiparallel orientation using middle linkers shorter than 12 amino acids. The new molecule called tandem Db (TandAb) was expressed in E coli as a highly stable bispecific and tetravalent dimer, which demonstrated...
increased valency and longer blood retention compared with scFv fragments and Dbs. With a size of approximately 110 kDa, TandAbs are smaller than an IgG molecule, which may enhance tumor penetration. However, their size is well above the renal threshold for first-pass clearance, offering a pharmacokinetic advantage over smaller bispecific formats. A TandAb platform has since been developed and is currently exploited by the company Affimed. One of their most advanced molecules, a CD30xCD16 TandAb called AFM13, is currently in a multicenter phase 2 clinical trial for the treatment of advanced relapsing/refractory Hodgkin lymphoma (NCT02321592). By specifically targeting CD16A, AFM13 is designed to recruit NK cells without interacting with neutrophils expressing CD16B, and is not affected by CD16A polymorphism that affects the Fc binding of conventional IgGs. Somehow surprisingly, the activation of NK cells was found strictly dependent on the presence of CD30+ target cells despite the TandAb bivalency for CD16A.

The same strategy has been applied for the recruitment of T cells with the anti-CD19xCD3 TandAb, AFM11, made of fully human binding domains, which are isolated as scFvs from a phage-display library. This molecule is currently in a preclinical development stage for the treatment of NHL. As for most CD3-targeting bsAbs, AFM11 exhibits potent cytotoxic activity in vitro with half-maximal effective concentration (EC50) values in the low- to subpicomolar range, with complete lysis of CD19+ tumor cells typically observed within 2 hours. Unexpectedly, despite its bivalency and its very high affinity for CD3 (0.7 nM), the binding of AFM11 to CD3 in the absence of a tumor cell appears to be insufficient to activate T cells. A side by side in vitro comparison with blinatumomab on CD19+ cells (NALM-6, a human pre-B cell line) demonstrated a higher potency for the TandAb AFM11. In vivo studies on a Burkitt lymphoma xenograft model demonstrated high tumor-cell killing and advantageous pharmacokinetic properties, suggesting that AFM11 might not require Fc fusions to target CD16A. The natural in vivo heterodimerization of Fab fragments was also used to create a very compact format of bsAb, devoid of artificial linkers. The so-called bsFabs (for bispecific Fab fragments) relies on the use of single-domain antibodies (also called nanobodies) derived from heavy-chain antibodies, naturally occurring antibodies devoid of light chains found in camels. In 2013, Rozan et al showed that an anti-CEAxCD16 bsFab could be efficiently produced by fusing anti-CD16 and anti-CEA nanobodies to the N-terminus of the human C\text{\textsubscript{ram}} and C\text{\textsubscript{c}} constant domains respectively, leading to a highly stable 50-kDa Fab-like bsAb able to elicit potent lysis of tumor cells by human NK cells at picomolar concentrations. This format was recently used to develop an anti-ERBB2xCD16 bsAb able to outperform trastuzumab, both in vitro and in vivo, on tumor cells expressing a low amount of ERBB2, thereby potentially enlarging the number of patients eligible for breast cancer immunotherapy.

\* C\text{\textsubscript{H}}/C\text{\textsubscript{C}} domains as a heterodimerization motif: tribodies and bispecific Fab fragments
bsAbs containing constant IgG domains have also been developed. In 2010, Mertens et al published a way to obtain trivalent antibody fragments (tribodies) by fusing a scFv at the C-terminus of C\text{\textsubscript{H}1} and C\text{\textsubscript{C}} constant domains of a Fab fragment (Figure 2). This format was later used by Glorius et al to create a bispecific anti-CD20xCD16 tribody by fusing 2 anti-CD20 scFv at the C-termini of an anti-CD16 Fab. Interestingly, the potency and efficacy of lysis obtained with the tribody was significantly higher than that triggered by rituximab. Compared with rituximab, the tribody demonstrated depletion of autologous B cells in ex vivo whole blood assays at a 100-fold lower antibody concentration, as well as in mice with a reconstituted, humanized hematopoietic system. Tribodies display interesting pharmacokinetic properties such as biodistribution profiles similar to those of IgG and higher tumor-accumulation rates. The company Biotecnol is currently exploiting this format to develop Tb535, an anti-CD3 Tribody™, directed against the oncofetal antigen 5T4, found in various subtypes of malignant mesothelioma and absent from normal tissue. Tb535 demonstrated low picomolar EC\text{\textsubscript{50}} values for cytotoxicity toward several human carcinoma cell lines (mesothelioma and others) in an in vitro assay using PBMCs from healthy human donors.

The natural in vivo heterodimerization of Fab fragments was also used to create a very compact format of bsAb, devoid of artificial linkers. The so-called bsFabs (for bispecific Fab fragments) relies on the use of single-domain antibodies (also called nanobodies) derived from heavy-chain antibodies, naturally occurring antibodies devoid of light chains found in camels. In 2013, Rozan et al showed that an anti-CEAxCD16 bsFab could be efficiently produced by fusing anti-CD16 and anti-CEA nanobodies to the N-terminus of the human C\text{\textsubscript{ram}} and C\text{\textsubscript{c}} constant domains respectively, leading to a highly stable 50-kDa Fab-like bsAb able to elicit potent lysis of tumor cells by human NK cells at picomolar concentrations. This format was recently used to develop an anti-ERBB2xCD16 bsAb able to outperform trastuzumab, both in vitro and in vivo, on tumor cells expressing a low amount of ERBB2, thereby potentially enlarging the number of patients eligible for breast cancer immunotherapy. In 2012, Kuo et al developed a straightforward bsAb format to target CD123+ leukemia cells by fusing an anti-CD123 scFv at the N-terminus of human IgG1 hinge-\text{C\text{\textsubscript{ram}}-C\text{\textsubscript{C}}} domains, followed by an anti-CD3 scFv at its C-terminus (Figure 2). Upon dimerization, this 160-kDa molecule, named Bif for bispecific immunofusion, is bispecific and tetravalent, and possesses an intact Fc portion allowing extended serum half-life and the ability to trigger ADCC. Bif shows cytolytic activities at low picomolar levels with effector:target ratios as low as 2. This molecule is bivalent for CD3, but the location of the anti-CD3 scFv at the C-termini of Bif reduces the affinity to CD3+ T cells by 2 orders of magnitude, which could help to prevent nonspecific T-cell activation. The company Trubion has developed a very similar bsAb format initially called SCORPION™. After their purchase by Emergent BioSolutions, that modular protein technology platform was renamed ADAPTIR™. In collaboration with MorphoSys, an anti-PSM\textsubscript{AX}CD3 bsAb named MOR209 or ES414 recently entered a phase 1 clinical trial evaluating the compound in patients with metastatic castration-resistant prostate cancer (NCT02262910).
bsAbs based on the dock-and-lock approach

The dock-and-lock (DNL) method was originally published in 2006 and represents a totally different way to create bsAbs. It relies on the spontaneous association of the 44-amino-acid peptide DDD2, derived from the regulatory subunit of human cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) with the 17-residue peptide AD2, derived from the anchoring domains (AD) of human A kinase anchor proteins (AKAPs). Upon association, 2 disulfide bonds are created, resulting in a covalent complex that is stable for more than a week at 37°C in human serum. This approach was recently developed to create T-cell retargeting bsAbs.33,34 (E1)-3s is a T-cell–redirecting trivalent bsAb, comprising an anti-CD3 scFv covalently linked to a stabilized dimer of a humanized Trop-2–targeting Fab (from murine mAb RS7). (E1)-3s mediated a highly potent T-cell lysis of NCI-N87 target cells in vitro. In vivo, (E1)-3s effectively induced T-cell–mediated killing of Trop-2–expressing pancreatic and gastric cancers, which was enhanced with interferon α (INFα).33

IgG-like formats

Chemically cross-linked full-length antibodies

The simplest way to create anti-CD3 bsAbs is to chemically cross-link already approved therapeutic IgGs such as anti-CD3 mAb OKT3 and anti-ERBB2 trastuzumab, creating a heterogeneous mixture with high valency and bispecificity. As early as 2001, Lum et al used such a preparation to arm a heterogeneous mixture with high valency and bispecificity. Indeed, patients receiving catumaxomab had a 4-fold increase in puncture-free survival compared with those receiving paracentesis therapy only. Catumaxomab is thus the first approved bsAb in the history of immunotherapy. Ertumaxomab, a second Triomab targeting ERBB2, has impressive preclinical data with total tumor eradication, but also with induction of immune protection. By early 2009, the results of a large international phase 2/3 pivotal study involving 258 patients demonstrated a statistically significant improvement of the primary end point, puncture-free survival, leading to the approval of the molecule by the European commission in April 2009 for the treatment of malignant ascites in patients with EpCAM-positive carcinomas in cases where standard therapy is not available or no longer feasible. Indeed, patients receiving catumaxomab had a 4-fold increase in puncture-free survival compared with those receiving paracentesis therapy only. Catumaxomab is thus the first approved bsAb in the history of immunotherapy. Ertumaxomab, a second Triomab targeting ERBB2, has also yielded impressive preclinical data and is currently in phase 1/2 trials for the treatment of patients with progressing ERBB2+ solid tumors (NCT01569412). A third Triomab, targeting CD20, called FBTA05 (or Lymphomun™ or Bi20), is under phase 1/2 clinical investigation for the treatment of chronic lymphocytic leukemia (CLL) and low- and high-grade NHL, in combination with donor lymphocyte infusions (NCT01138579).

Intact IgG with engineered Fc

Several published approaches allow the generation of intact mAbs. The knobs-into-holes principle is a well-described Fc heterodimerization technology that consists of introducing complementary mutations (replacing a small amino acid with a larger one [*“knob”*] and vice versa [*“hole”*]) in each CH3 domain. The light-chain mispairing issue has also been solved via the use of common light chains, or more elegantly via the CrossMab technology which avoids nonspecific light-chain mispairing by exchanging C\textsubscript{H}3 and C\textsubscript{L} constant domains in

Y-shaped bispecific IgG

Triomabs

Because they rely on an adaptation of the conventional hybrid hybridoma technology, Triomabs represent the simplest format of IgG-like bsAbs. In 1995, Lindhofer et al published a paper describing a major improvement of the classical quadroma approach to produce bsAbs. By using an original subclass combination (mouse IgG2a and rat IgG2b), they demonstrated a preferential species-restricted heavy/light-chain pairing, in contrast to the random pairing in conventional mouse/mouse or rat/rat quadromas, as well as the use of sequential pH elution on protein A to easily separate the desired bsAb from the parental mAb. Surprisingly, the resulting hybrid rat/mouse Fc portion efficiently interacted with activating human Fc receptors (FcyRIIa and FcyRIIb), but not with inhibitory ones (FcyRIIB), thereby reaching the goal that other groups had hoped to achieve using human Fc engineering. The investigators used this approach to create an anti-CD3xEpCAM bsAb, and demonstrated that this antibody was capable of binding to target cells and human T cells, but was also capable of activating dendritic cells (DCs), inducing NK-dependent ADCC and stimulating tumor-cell phagocytosis by macrophages. In short, this Fc adds 2 crucial functions to regular anti-CD3 x target bsAbs: additive tumor-killing capabilities through the efficient recruitment of macrophages and NK cells, and, most importantly, efficient costimulation of T cells through direct contact with accessory cells, such as macrophages and DCs or cytokine secretion. The most advanced Triomab, an anti-EpCAMxCD3 bsAb called catumaxomab, has impressive preclinical data with total tumor eradication, but also with induction of immune protection. By early 2009, the results of a large international phase 2/3 pivotal study involving 258 patients demonstrated a statistically significant improvement of the primary end point, puncture-free survival, leading to the approval of the molecule by the European commission in April 2009 for the treatment of malignant ascites in patients with EpCAM-positive carcinomas in cases where standard therapy is not available or no longer feasible. Indeed, patients receiving catumaxomab had a 4-fold increase in puncture-free survival compared with those receiving paracentesis therapy only. Catumaxomab is thus the first approved bsAb in the history of immunotherapy. Ertumaxomab, a second Triomab targeting ERBB2, has also yielded impressive preclinical data and is currently in phase 1/2 trials for the treatment of patients with progressing ERBB2+ solid tumors (NCT01569412). A third Triomab, targeting CD20, called FBTA05 (or Lymphomun™ or Bi20), is under phase 1/2 clinical investigation for the treatment of chronic lymphocytic leukemia (CLL) and low- and high-grade NHL, in combination with donor lymphocyte infusions (NCT01138579).
the Fab of one-half of the bsAbs (see reference 4 for detailed review). The use of such innovative formats for effector-cell retargeting is not yet well documented in the literature. The company Xencor has used structure- and sequence-based approaches to design Fv variants that preferentially heterodimerize to produce 3 anti-CD3 bsAbs targeting CD123, CD38, and CD20, all of them being in preclinical stage. Using an undisclosed technology, the company Regeneron Pharmaceuticals has generated an anti-CD20xCD3 bsAb, named REGN1979, which recently entered a multicenter phase 1 clinical trial for the treatment of patients with NHL and CLL (NCT02290951).

Conclusion

The journal Science has chosen cancer immunotherapy as “Breakthrough of the Year 2013,” mainly because of the clinical successes recorded with immunomodulatory mAbs such as anti–cytotoxic T-lymphocyte antigen 4 (CTLA-4), anti–programmed cell death protein 1 (PD-1), anti–programmed cell death-ligand 1 (PD-L1), and other promising approaches such as chimeric-antigen-receptor T cells and antibody drug conjugates. It might well be that, in the end, bi- and multispecific antibodies will have just as much impact as those approaches, if not more. The long-awaited results of ongoing clinical trials will surely tell.


Keywords: bispecific antibodies; Bispecific T-cell Engager, cancer immunotherapy; CD3; CD16; Dual-Affinity Re-Targeting; FcγRIIIA

Des anticorps bispécifiques comme nouvelles immunothérapies contre le cancer

Une classe d’anticorps bispécifiques ciblent simultanément un antigène associé à une tumeur et un récepteur activateur situé à la surface de cellules effectrices, les cellules T ou cellules NK. Ces dernières années, le développement de l’ingénierie des anticorps a favorisé l’émergence de plusieurs nouveaux formats d’anticorps bispécifiques conduisant à des résultats précliniques remarquables in vitro et in vivo. Un grand nombre de ces formats font actuellement l’objet de recherches cliniques approfondies. De plus, fin 2014, la FDA (Food and Drug Administration US) a accordé, pour la première fois de l’histoire, une autorisation de mise sur le marché à un anticorps bispécifique recombinant ciblant les cellules T appelé le blinatumomab (BLINCYTO™) pour le traitement de la leucémie lymphoblastique aiguë en rechute ou réfractaire à précursores de cellules B. Cet article analyse les différents formats d’anticorps conçus pour générer de telles immunothérapies en insistant spécialement sur les molécules en recherche clinique. Plusieurs de ces molécules devraient radicalement améliorer le traitement de nombreuses pathologies malignes.
Chimeric antigen receptor technology: a breakthrough in immuno-oncology

by D. Campana, Singapore

Chimeric antigen receptors (CARs) can redirect the specificity of immune cells. An antibody-derived single-chain fragment variable (scFv) provides specific capacity to bind a surface antigen expressed by cancer cells. The scFv is linked, via hinge and transmembrane protein segments, to signaling domains that trigger T-cell activation when the CAR binds to its cognate antigen. Contemporary CARs typically contain a primary signaling molecule such as CD3ζ, and one or two costimulatory molecules, such as CD28 and/or 4-1BB (CD137). Costimulation sustains T-cell proliferation and suppresses activation-induced cell death. CAR-engineered T cells can exert powerful cytotoxicity against cancer cells in vitro and in animal models. Results of recent clinical studies have demonstrated the remarkable potential of this technology, with durable remissions achieved in patients with refractory B-cell leukemia and lymphoma targeted with anti-CD19 CARs. Whether such responses will also be seen in other malignancies is still unknown. Infusion of CAR-engineered T cells can have serious side effects, such as cytokine release and tumor lysis syndromes, and “on-target off-tumor” activity caused by expression of tumor antigens in normal cells. These issues must be addressed and better ways for large-scale production of CAR T cells must be developed. Ultimately, the generation of highly optimized “living drugs” that can be administered on demand with predictable activity should lead to the incorporation of CAR T cells into mainstream cancer treatment.

Medicographia. 2015;37:280-286 (see French abstract on page 286)
If one could redirect autologous T cells towards tumor cells, then it might be possible to have graft vs leukemia without GvHD. This was made possible by the advent of chimeric antigen receptors (CARs).

**Your CAR is here**

A CAR is an artificial multimolecular receptor. Its specificity derives from a single-chain fragment variable (scFv) of an antibody, ie, a fusion protein containing the portions of an antibody specifically binding to a target antigen connected with a short peptide. The scFv is linked to a transmembrane domain that ensures expression on the cell membrane; a hinge region is generally placed between the scFv and the transmembrane domain to give flexibility to the CAR and facilitate antigen binding and signal transduction. The power house of the receptor is in the intracellular portion and consists of a signaling domain, typically a T-cell receptor (TCR)–associated signaling molecule, such as cluster of differentiation 3ζ (CD3ζ). Thus, scFv ligation to its unique antigen triggers signal transduction, similar to the one triggered by the TCRs normally expressed on T lymphocytes when they encounter a viral peptide. This results in T-cell activation, proliferation, and cytotoxicity (Figure 1). Therefore, by expressing a CAR in T lymphocytes, one can instantaneously generate a massive cohort of immune cells reacting against a tumor.

The concept of CAR (also referred to as “chimeric T-cell receptor,” “chimeric antibody/T-cell receptor,” or “T-body”) was first reported 25 years ago by Zelig Eshhar and his colleagues working at the Weizmann Institute of Science in Israel. In their initial studies, a prototype CAR was made by splicing the antigen-binding regions of an antibody against 2,4,6-trinitrophenyl hapten to a TCR. The construct was transfected into a mouse T-cell hybridoma that could kill cells expressing hapten triphenyl. While the constructs were developed with the intention of studying T-cell activation mechanisms rather than developing a novel cancer treatment; the pioneering article already states that “This approach can be exploited, for example, to direct cytotoxic T lymphocytes to kill tumor or virally infected cells.” Later, the group refined the original prototype by linking scFv to CD3ζ (the subunit of the TCR/CD3 complex that transduces signals) or FcRγ chains, containing signaling cassettes known as immunoreceptor tyrosine-based activation motifs (ITAMs). Together with investigators at the National Cancer Institute in Bethesda, they published a study that tested the CAR concept in a more realistic experimental scenario. This new CAR was made with a scFv that reacted against a folate receptor overexpressed by ovarian cancer cells and was expressed in CD8+ tumor-infiltrating lymphocytes (TILs). Expanding the range of targetable antigens, a CAR reacting with the tumor-associated antigen human epidermal growth factor receptor 2 (HER2/neu) was reported soon after.

The portfolio of antigens targetable by CARs has progressively increased. Those expressed by solid tumors included tumor-associated glycoprotein 72 (TAG-72), and epithelial glycoprotein 2 (EGP-2), both overexpressed in multiple carcinomas, as well as GD-2, expressed in neuroblastoma, sarcomas, and melanoma. CARs directed against antigens expressed in lymphomas and leukemias were also reported, including CD30, expressed in Hodgkin and anaplastic large cell lymphoma, CD20, expressed in B-cell non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (CLL), and CD33, expressed in acute myeloid leukemia (AML).

**New generations of CARs**

During immune responses, TCR activation alone cannot provide a sufficiently robust stimulus to T lymphocytes; without participation of other “costimulatory” receptors, T-cell activation is short-lived, ultimately leading to T-cell unresponsiveness and/or apoptosis. One of the most extensively studied costimulatory molecules in T lymphocytes is CD28, which...
interacts with its ligands B7-1 (CD80) and B7-2 (CD86) expressed by antigen-presenting cells. Another costimulatory molecule is 4-1BB (CD137), which also increases lymphocyte activation and supports proliferation (reviewed in reference 15). Some studies suggested that 4-1BB stimulation could elicit more effective antitumor responses than those provoked by CD28, and that it preferentially expanded memory T cells.15

In general, costimulatory ligands are poorly expressed by tumor cells.16,17 Therefore, stimulation via a CAR containing only CD3ζ as a stimulatory module might result in unpredictable activation, depending on the degree of expression of costimulatory ligands in target cells. Elegant experiments performed by Brentjens et al18 illustrated this concept well. They found that in immunodeficient mice engrafted with tumor cells, the antitumor activity of T lymphocytes expressing an anti-CD19 CAR was considerably higher if the target cells were induced to overexpress the CD28 ligand, CD80.

A solution to the problem of variable costimulatory capacity by tumor cells is to integrate the costimulatory signal directly into the CAR.19-22 CARs bearing costimulatory signaling domain are designated as “second generation” to distinguish them from the “first-generation” CARs that could only deliver a primary, ITAM-derived, signal (Figure 2). Second-generation CARs provoke a much more reliable and robust T-cell stimulation than their predecessors. Finney et al19 found that CD28-containing CARs triggered higher interleukin 2 (IL-2) production as compared with constructs containing only CD3ζ without CD28; placing CD28 proximal to the cell membrane led to more efficient CAR expression. Maher et al20 reported that T lymphocytes expressing a CAR directed against prostate-specific membrane antigen were considerably more effective at triggering cytokine secretion, and proliferation, and tumor cell killing if CD28 was added to CD3ζ.

Because 4-1BB also plays an important costimulatory role in T cells, we constructed anti-CD19 CAR signaling via CD3ζ and added a 4-1BB signaling domain.17 The receptor contained hinge and transmembrane domains derived from human CD8κ; all components were joined in a unique chimeric sequence. The expression of this CAR achieved in peripheral blood lymphocytes by retroviral transduction was high (median, 64%) and the addition of 4-1BB did not affect levels of expression.17 We found that this CAR could also be expressed by electroporation of the corresponding messenger RNA.23,24 When cocultured with CD19+ leukemic cells for 24 hours, T cells expressing the anti-CD19-BB-ζ CAR produced about 5 times more IL-2 than T cells expressing an equivalent receptor lacking 4-1BB.17 T lymphocytes expressing the 4-1BB CAR expanded for 3 weeks or more when cultured in the presence of CD19+ target cells without exogenous IL-2, while T cells transduced with the CAR lacking 4-1BB did not grow and survived for less than 2 weeks.17 The addition of 4-1BB also increased tumor cell killing, and the difference with receptors lacking 4-1BB was particularly evident at a low effector:target ratio in cultures extended to 5 days instead of the standard 4-hour assays.17 Research from other laboratories also demonstrated that 4-1BB contributes significantly to CAR function.19-21

**What is the best CAR?**

At present, it is not clear what CAR configuration is optimal, although it seems unquestionable that “second-generation” constructs are more potent than “first-generation” constructs.

We found that 4-1BB induced higher production of IL-2, while CD28 induced higher production of interferon γ (IFNγ).16,17 Increased production of IFNγ by CD28 was also reported by others.25,28 We could not detect differences in cytotoxicity mediated by the two CARs, but observed that the 4-1BB CAR induced better expansion of T cells in the presence of low concentrations of IL-2.16,17

Higher proliferation with the 4-1BB CAR was confirmed by Milone et al25 in immunodeficient mice. So-called “third-generation” CARs contain more than one costimulatory domain; it is not clear whether this will produce a proportionally more potent receptor. Some studies indicated that adding both 4-1BB and CD28 to a CAR increased cytokine production, proliferation, and cytotoxicity as compared with a single costimulus.19 Others, however, could not detect clear differences between single- and dual-costimulation CARs.16 Data on CARs with other costimulatory molecules (OX40, CD27, etc) are not extensive.
When building a CAR, cloning its various components is only the beginning; putting them together to maximize CAR expression and function requires much attention. As initially described for CD28,10 our anti-CD19-BB-ζ receptor requires the 4-1BB to be placed proximal to the transmembrane domain; we also modified the hinge and transmembrane portion derived from CD8α to allow optimal delivery of 4-1BB signaling.15,17 Simply inserting 4-1BB into any CAR might not produce the same results without attention to these details. The same probably applies to CD28 and to “third-generation” CARs. To this end, Kochenderfer et al29 showed that combining CD28 and 4-1BB actually decreased IL-2 production as compared with CD28 alone, but the transmembrane domains of the compared CARs were different (CD8 vs CD28). Along the same lines, Haso et al30 reported that CARs with single 4-1BB or CD28 domains were better than those containing both, but again, transmembrane domains were different as were levels of expression for different CARs. Another important variable that can influence results of CAR comparisons is the type of test used to assess function. For example, Finney et al31 did not observe any advantage when they added 4-1BB to their CAR, but T cells were tested only in short-term assays. Indeed, we found that the superior proliferation and cytotoxicity of 4-1BB CARs became obvious only when the experiments were extended for days and performed at low effector:target ratios.15,17 Finally, the type of antigen targeted may also play a role: in addition to scFv affinity,30,31 the length of hinge domain can also be a critical factor, as it has been shown for CARs targeting ROR131 and MUC1.32

**CAR-engineered T lymphocytes in the clinic**

The typical process for preparing CAR-engineered T cells consists of collecting blood from the patient via leukapheresis, followed by T-cell activation and expansion for about 10 days using anti-CD3 or anti-CD3 plus anti-CD28 stimulation (either with soluble antibodies or antibodies bound to a solid phase) and IL-2. During the culture, cells are exposed to retroviral or lentiviral supernatant containing the CAR construct in a viral vector. In future trials, additional genes might be included in the constructs, such as those encoding proteins that can facilitate the elimination of engineered cells to limit adverse effects, or allow the infusion of allogeneic cells. At the completion of the culture, cells are washed, concentrated, and infused. The cell product might be cryopreserved before infusion to allow for sterility and potency testing to take place. Before infusion, the patient may receive lymphodepleting chemotherapy (eg, fludarabine and cyclophosphamide) to facilitate the expansion of the infused T cells (Figure 3).33 The cell preparation typically takes place in facilities that work under Current Good Manufacturing Practice (CGMP) regulations enforced by the US Food and Drug Administration (FDA), or similar regulations imposed by equivalent authorities in other countries.

In the first reported study using CAR-engineered T cells, 14 patients with metastatic ovarian cancer received T cells expressing a first-generation CAR against an α-folate receptor.34 The infused cells were initially present in large numbers, but then declined and became nearly undetectable even by polymerase chain reaction (PCR) after 1 month; no antitumor effect was observed.35 In another early study, T cells expressing a first-generation CAR against carboxy anhydrase IX were administered to 12 patients with metastatic renal cell carcinoma.36 Levels of infused T cells in blood peaked at around day 6 and were detectable after about 1 month by PCR. Liver toxicity (possibly due to “on-target off-tumor” activity) was observed, but no antitumor activity.35 Because both of these studies used a first-generation CAR, lack of vigorous T-cell expansion and persistence might have contributed to the lack of antitumor activity, although it is possible that other factors may have played a role. Neither study used lymphodepletion prior to T-cell infusion, and T-cell activation ex vivo was performed without costimulation.

Among other early studies with first-generation constructs, an anti-GD2 CAR was used to treat 11 patients with neuroblastoma and active disease at the time of infusion, producing remission in three of these patients.36,37; CAR T cells persisted for up to 3.6 years.37 No responses were seen in four patients with B-cell NHL who received T cells expressing anti-CD19 or anti-CD20 CARs; short persistence of the infused cells was noted, with evidence of immune rejection, which might have been related to the incorporation of selection and suicide genes in the construct.38

**Figure 3.** Preparation of chimeric antigen receptor (CAR)–engineered T lymphocytes for clinical use. Peripheral blood is obtained from the patient, eg, by leukapheresis. In a facility operating under Current Good Manufacturing Practice (CGMP) guidelines, T lymphocytes are activated and transduced with viral supernatant and viral transduction procedures are performed. After approximately 10 days from the beginning of the culture, CAR-engineered T cells are reinfused in the patients. Patients often receive lymphodepleting chemotherapy during ex vivo cell processing to facilitate the engraftment of the infused cells.
Much more encouraging results were obtained in patients with B-cell malignancies using T cells engineered with second-generation CARs. In 2010, Kochenderfer et al reported the case of a patient with follicular lymphoma and progressive disease treated with T cells expressing an anti-CD19 CAR signaling via CD3ζ and CD28. T cells were infused after lymphodepleting chemotherapy and a major disease regression with remission lasting 32 weeks was observed. There was also concomitant B-cell aplasia and hypogammaglobulinemia. In a recent update including 15 patients with NHL or CLL, complete remission was achieved in 8 of the 15 patients and a partial remission in 4, with 3 remissions in patients with diffuse large B-cell lymphoma continuing beyond 9-22 months. This group also reported results of infusing CAR-modified T cells in patients with B-cell malignancies post-allogeneic stem cell transplant, where the T cells were from the stem cell donor. While disease was resistant to infusion of unmodified donor lymphocytes, 3 of the 10 patients had disease regression following anti-CD19 CAR infusion. Interestingly, none of the 10 patients developed GvHD. In a recent report, this CAR was used to modify autologous T cells in 20 children and young adults with acute lymphocytic leukemia (ALL); 14 achieved complete remission, 12 with minimal residual disease negativity (Table I).

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of patients enrolled</th>
<th>Number of patients achieving complete remission</th>
<th>Number of patients with negative MRD</th>
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</thead>
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<tr>
<td>Davila et al.</td>
<td>16</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Maude et al.</td>
<td>30</td>
<td>27</td>
<td>22*</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>20</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>55 (83%)</td>
<td>46 (70%)</td>
</tr>
</tbody>
</table>

Table I. Response to anti-CD19 chimeric antigen receptor (CAR) in patients with acute lymphocytic leukemia (ALL).

*Minimal residual disease (MRD) studies were not performed in 2 of the 27 patients who achieved complete remission. Based on data from references 42, 45, and 47.

In 2011, using an anti-CD19 CAR costimulating via 4-1BB, Porter et al reported major responses in all 3 patients with treated CLL, with durable complete remission in 2 patients. Infused T cells expanded and persisted for at least 6 months. As expected, there was B-cell aplasia and hypogammaglobulinemia. Grupp et al used the same anti-CD19 to treat patients with relapsed or refractory ALL. In their most recent report, a complete remission was achieved in 27 of 30 patients, with a 6-month event-free survival rate of 67% and an overall survival rate of 78% (Table I). Three patients relapsed with CD19-negative leukemia.

Anti-CD19 CARs costimulating via CD28 were used in other studies. Brentjens et al reported responses in 3 of 4 patients with chemotherapy-refractory CLL. In a study from this group using the same CAR to treat 16 patients with relapsed or refractory ALL, a complete response was achieved in 14 patients, with molecular remission in 12, allowing patients to receive allogeneic hematopoietic stem cell transplants. Cruz et al expressed the anti-CD19 CAR in virus-specific, donor-derived T cells and infused them in 6 patients with relapsed CLL or ALL after hematopoietic stem cell transplant. There was no GvHD and cells persisted for several weeks in blood. Responses were seen in 2 of 6 patients.

A CD20 CAR, this time a "third-generation" construct costimulating via both CD28 and 4-1BB, was used to treat 4 patients with relapsed indolent B-cell and mantle cell NHL. Response is difficult to evaluate as 2 patients remained progression-free for 12 and 24 months, but did not have evaluable disease before infusion. The third patient had a partial remission with relapse 12 months later. Modified T cells were detected by PCR at tumor sites and up to 1 year in peripheral blood. CARs can also be expressed in natural killer cells and clinical studies with such cells are ongoing.

Areas for improvement

Ehrlich’s concept of "magic bullets" that specifically bind to certain cells and eliminate them while sparing others was formulated at the dawn of the 19th century and inspired legions of oncologists to identify such agents. This seemed to be embodied by monoclonal antibodies, but clinical results, albeit exciting, have rarely met Ehrlich’s criteria of "therapia sterilisans magna," which in oncology requires complete eradication of tumor. The excitement around CAR-engineered T cells is that they hold promise to achieve such a goal. The clinical data summarized here demonstrated dramatic and durable responses in patients with leukemia and lymphoma resistant to contemporary intensive chemotherapy and stem cell transplant, and therefore are extremely encouraging.

Although effective, CAR technology needs improvement. Successful studies with CARs have been performed in patients with lymphoid malignancies and it is not clear whether a similar success can be achieved in other types of cancer. To this end, there is a relative paucity of good targets and much research is needed. An elegant approach that may be helpful in this respect is that of using dual CARs that are either active or inhibited depending on the simultaneous expression of two antigens. Infusion of CAR-engineered T cells can have serious side effects, such as cytokine release and tumor lysis syndromes. Developing ways to better control T-cell activation would be useful. Also, it has been shown that ALL treated with anti-CD19 CARs can relapse as a CD19-negative ALL. Targeting multiple antigens simultaneously might help preventing such relapses. We developed a new chimeric receptor, CD16V-BB-ζ, whose specificity is directed by immunotherapeutic antibodies, instead of scFv; hence, one can target multiple antigens with one single receptor. In B-cell malignancies, for example, the CD16V-BB-ζ receptor would...
allow T-cell therapy simultaneous targeting CD19, CD20, and CD22 with existing humanized antibodies. Moreover, T-cell therapy simultaneously targeting CD19, CD20, and CD22 achieves this.

References
Les récepteurs antigéniques chimériques (CAR) peuvent réorienter la spécificité des cellules immunitaires. Une variante d’un fragment d’anticorps simple chaîne (scFv) permet de se lier spécifiquement à un antigène de surface exprimé par les cellules cancéreuses. Le scFv se lie, via des segments protéiques charnières et transmembranaires, aux domaines de signalisation qui déclenchent l’activation des cellules T lorsque le CAR se lie à son antigène apparenté. Les CAR actuels contiennent généralement une première molécule de signalisation comme un CD3ζ et une ou deux molécules co-stimulantes, comme le CD28 et/ou le BB1-4 (CD137). La co-stimulation maintient la prolifération des cellules T et supprime la mort cellulaire induite par l’activation. Les cellules T conçues avec des CAR peuvent être très cytoxiques contre les cellules cancéreuses en vitro et dans les modèles animaux. D’après des études cliniques récentes, le potentiel de cette technologie est remarquable, des rémissions durables ayant été observées chez des patients atteints de leucémies à cellules B réfractaires et de lymphomes ciblés par CAR anti-CD19. On ne sait toujours pas si de telles réponses peuvent être obtenues dans d’autres pathologies malignes. Les effets secondaires des perfusions de cellules T conçues avec des CAR peuvent être sévères, comme la libération de cytokine et les syndromes de lyse tumorale et l’activité « sur cible/hors tumeur » provoquée par l’expression d’antigènes tumoraux dans des cellules normales. Ces questions doivent être abordées et il faut développer de meilleures voies de production de cellules T CAR à grande échelle. Enfin, la génération de « médicaments vivants » très optimisés, administrables sur demande avec une activité prévisible, devrait permettre d’intégrer les cellules T-CAR au traitement habituel du cancer.

Keywords: acute lymphocytic leukemia; chimeric antigen receptor; chronic lymphocytic leukemia; costimulatory molecule; non-Hodgkin lymphoma; T lymphocyte
Trials investigating novel molecular therapies and biomarker profiles have required significant changes to the traditional paradigm for clinical drug development. Furthermore, there are now major efforts in cancer centers across the world to establish routine molecular profiling services for cancer patients. These efforts help to identify groups of patients with particular molecular aberrations required for clinical trial entry, and also permit researchers to study correlations between molecular profiles and clinical outcomes.

There is great variability in tumor biology among cancer patients, determining response to treatments and clinical outcomes. Much of this variability can now be explained by differences in the biomarker profiles between tumors. This review uses breast cancer as a focus for discussing contemporary biomarker-driven approaches in cancer therapy. The most well-established biomarkers in breast cancer are tumor expression of the estrogen receptor (ER) and receptor tyrosine-protein kinase erbB-2 (HER2), high expression levels of which open up the possibility of ER- and HER2-directed therapies, respectively. Additional cancer drivers have been identified in recent decades, including aberrations in the PI3K-AKT-mTOR pathway (phosphatidylinositol 3-kinase; protein kinase B; mammalian target of rapamycin), fibroblast growth factor receptor, cell-cycle control, tumor angiogenesis, and immune control. To tackle these cancer drivers, a large number of novel molecular targeted therapies in breast cancer are in clinical development. It is increasingly apparent that most molecular targeted therapies will only work in biomarker-defined subsets of patients. This has required the development of new clinical trial methodologies, including “basket” and “umbrella” trial designs to match molecular therapy to the patient’s biomarker profile, as well as novel Bayesian adaptive approaches. Key to the success of this approach will be repeated sampling of patient tumors over time, which can be difficult if invasive tumor biopsies are required, but may be transformed by new “liquid biopsy” technologies to measure circulating free DNA and circulating tumor cells.

No 2 cancer patients are exactly the same. Even patients with cancers that are the same size and stage, and that are very similar at the microscopic level using standard pathological and immunohistochemical criteria, can have dramatically different natural histories and responses to therapy. Clinicians know this only too well. Indeed, one of the central questions of oncology is why 2 similar patients treated in the same way can have such different clinical outcomes. It is now well established that this variation can be largely explained by differences in the molecular or biomarker profiles between different cancers. This review focuses on breast cancer as a paradigm for contemporary biomarker-driven approaches in cancer therapy.

Medicographia. 2015;37:287-295 (see French abstract on page 295)
Breast cancer is not one but many diseases, each defined by its biomarker profile

The best established breast cancer biomarkers are estrogen receptor (ER) expression and receptor tyrosine-protein kinase erbB-2 (HER2) expression. It is striking that with all the advances in molecular biology and genomic technologies in the last 2 years, ER and HER2 are the only universally accepted biomarkers used in clinical practice for the management of breast cancer patients.

However, there remain large differences in clinical outcomes between patients with the same ER and HER2 status. With the development of novel genetic technologies over the last 20 years, a number of genomic classifiers have been developed to help explain this clinical variability.

The first generation of breast cancer classifiers examined the correlation of gene expression with clinical outcomes, using complementary DNA (cDNA) microarray and polymerase chain reaction (PCR)-based techniques. This work was pioneered by the Stanford group, who used unsupervised hierarchical clustering of gene-expression profiles to discover 5 new “intrinsic subtypes” of breast cancer: luminal A, luminal B, normal breast-like, HER2-positive, and basal-like cancers.1

There have since been a number of efforts to standardize and commercialize gene-expression–based breast cancer assays. The MammaPrint test is a 70-gene expression signature originally developed from a series of patients who had undergone definitive surgery, but no systemic therapy, and for whom long-term follow-up was available2; it was approved by the US Food and Drug Administration (FDA) for prognostic prediction in 2007. Oncotype DX is a 21-gene, real-time PCR-based assay which was initially developed to predict the risk of cancer recurrence for women treated for stage I and II hormone-receptor–positive, lymph-node–negative, invasive breast cancer by surgery and 5 years of adjuvant tamoxifen.3

The largest breast cancer genomic study to date has been performed by the METABRIC group (Molecular Taxonomy of Breast Cancer International Consortium), combining gene copy number and expression from 2000 primary breast cancers, with long-term clinical follow-up.4 Analysis of the combined DNA-RNA profiles revealed that breast cancer can be classified into 10 different subgroups, each with different clinical outcomes (Figure 1).5 This new taxonomy of breast cancer has now been robustly validated in 7500 external samples.6 In contrast to the early gene-expression–based studies discussed above, this included 7 distinct subgroups of ER-positive breast cancer, and separation of triple-negative can-
Molecular targeted therapies for breast cancer

The discovery and validation of novel drug targets in breast cancer has led to a large number of corresponding drug-discovery programs. Selected molecular targeted therapies for breast cancer in clinical development or recently approved are shown in Table I. 8-17

Tumor heterogeneity in space and time – the need for repeat tumor sampling

In the past, comprehensive studies of tumor heterogeneity at the molecular level have been constrained by the lack of available biopsy specimens. Clinicians naturally wanted to avoid unnecessary invasive biopsy procedures, particularly if this didn’t change patient management.

There is now, however, a recognition that repeat tumor biopsy at the time of metastatic relapse can be crucial to optimization of patient care, since ER and HER2 status can change over time. In one meta-analysis, for example, it was found overall that 14% of patients were reclassified from ER-negative to ER-positive and 5% of patients were reclassified from HER2-negative to HER2-positive, opening up the possibility of new endocrine therapy and anti-HER2-therapy options, respectively.7 While ER- and HER2-directed therapies have without question transformed the clinical outcomes for patients with breast cancer, painstaking translational research over recent years has uncovered additional key drivers of malignant progression. These include nodes in the PI3K-AKT-mTOR pathway (phosphatidylinositol 3-kinase; AKT or protein kinase B; mammalian target of rapamycin); receptor tyrosine kinases (eg, fibroblast growth factor receptor [FGFR]; cell-cycle control (eg, cyclin-dependent kinases 4 and 6 [CDK4/6]); DNA damage repair pathways (eg, poly(ADP-ribose) polymerase [PARP]); and immune checkpoints.

Table I. Selected molecular targeted therapies in clinical development or recently approved for the treatment of breast cancer.

<table>
<thead>
<tr>
<th>Drug target</th>
<th>Examples</th>
<th>Phase of clinical development</th>
<th>Trial reference</th>
</tr>
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<tbody>
<tr>
<td>HER2</td>
<td>Antibodies</td>
<td>Pertuzumab, T-DM1</td>
<td>Approved 2012, Approved 2013</td>
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<td></td>
<td>Antibody-drug conjugate</td>
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<td>PI3K</td>
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<td>Eribulin (GDC-0068), BKM120</td>
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<td>mTORC1 + mTORC2</td>
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<td>Immunotherapy</td>
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</table>

Abbreviations: AKT, also known as protein kinase B; CDK, cyclin-dependent kinase; ER, estrogen receptor; HER2, receptor tyrosine-protein kinase erbB-2; FGFR, fibroblast growth factor receptor; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; oral SERDs; oral selective estrogen receptor degraders; PARP, poly(ADP-ribose) polymerase; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PGM, Personal Genome Machine; PI3K, phosphatidylinositol 3-kinase; TKI, tyrosine kinase inhibitor.
tory approval for pertuzumab in 2012. Another drug approved in 2013 for the treatment of HER2-positive metastatic breast cancer was the antibody drug conjugate, trastuzumab emtansine (T-DM1). T-DM1 binds the trastuzumab antibody to a cytotoxic maytansine derivative (DM1), using a stable linker. This specifically targets cytotoxic drug delivery to HER2-over-expressing cells, thereby minimizing cytotoxic exposure to normal tissues and improving therapeutic index. FDA approval for T-DM1 was granted following the phase 3 EMILIA trial (NCT00829166; not an acronym), which randomized patients with HER2-positive advanced breast cancer, who had received prior taxane- and trastuzumab-based therapy, to either T-DM1 or lapatinib + capecitabine. In this second/third-line population, T-DM1 demonstrated improved efficacy (median progression-free survival [PFS], 9.4 months vs 6.4 months, respectively), and reduced toxicity.

**PARP inhibitors**
A further important group of molecular targeted therapies are those that target cancer-cell defects in DNA repair. Preeminent in this category are inhibitors of the enzyme PARP. Ten years ago, preclinical data suggested that cancer cells with impaired DNA repair via the homologous recombination pathway were exquisitely sensitive to PARP inhibition, via so-called "synthetic lethality." Early phase clinical trials of PARP inhibitors suggested a high degree of antitumor activity for these agents, particularly against tumors with breast cancer gene (BRCA) mutations. Hopes were high that these drugs might reach a wider group of patients than merely those with BRCA mutations, including high-grade serous ovarian cancer, triple-negative breast cancer, and other sporadic cancers with a "BRCAness" phenotype. However, the clinical development programs of PARP inhibitors encountered a number of serious challenges. First, the PARP-inhibitor field was damaged by the results from the iniparib development program; partly by a surprising negative phase 3 trial when iniparib was added to standard chemotherapy, and subsequently by the revelation that iniparib wasn’t a PARP inhibitor after all. Second, the level of antitumor activity for non-BRCA1-mutant tumors was disappointing, including data in triple-negative breast cancer. However, in the last few years, the PARP-inhibitor field has experienced a resurgence, with clinical development focused on BRCA1-mutant tumors. In early 2015, regulatory approval for olaparib was granted for the maintenance treatment of BRCA1-mutant ovarian cancer. Ongoing clinical research strategies are focused on the development of predictive biomarkers for defective homologous recombination and the use of PARP inhibitors in combination with other agents, for example PI3K pathway inhibitors.

**VEGF**
It is now well understood that a nascent tumor cannot grow beyond a tiny size without creating a network of blood vessels to supply it with nutrients and to take away waste products, a process called angiogenesis. This hallmark trait of cancer is mediated by a number of factors including vascular endothelial growth factor (VEGF) and its receptor. A number of drugs have been developed to inhibit VEGF, including the monoclonal antibody bevacizumab, which gained conditional FDA approval following the results of the E2100 trial. In this study, the investigators found that the addition of bevacizumab to paclitaxel for the first-line treatment of metastatic breast cancer yielded an improved overall response rate [36.9% vs 21.2%; \(P<0.001\)] and increased median PFS (11.8 vs 5.9 months; \(P<0.001\)). However, there proved to be no overall survival difference for the addition of bevacizumab and, in fact, treatment was associated with an increased risk of potentially dangerous toxicities, including hypertension, bleeding, and gastrointestinal perforation. Thus, in December 2010, the FDA recommended removing the first-line metastatic breast cancer indication from the approval for bevacizumab. In the last few years, this conclusion has been bolstered by negative clinical trial results for bevacizumab in the adjuvant breast cancer setting. Current clinical research efforts include those focused on identifying predictive biomarkers for maximum bevacizumab benefit, for example the ARTemis trial (Avastin Randomized Trial with neo-adjuvant chemotherapy for patients with early breast cancer [NCT01093235]).

**PI3K-AKT-mTOR inhibitors**
The PI3K-AKT-mTOR pathway is one of the most commonly deregulated in human cancer. For example, up to 40% of breast cancers are found to bear PIK3CA mutations. Activation of the pathway in clinical cancer samples is associated with poor prognosis in multiple tumor types, and functional studies in preclinical models suggest that the pathway’s activation can promote malignant progression through increased proliferation, increased cell survival, and promotion of invasion and metastasis. Furthermore, the nodes in the pathway are druggable targets, which has led to a huge number of inhibitors in clinical development. Selected PI3K, AKT, and mTOR inhibitors are listed in Table 1 and are reviewed more comprehensively elsewhere.

First-generation “rapalog” mTOR complex 1 (mTORC1) inhibitors were the first agents in this group to reach the clinic and in 2012, everolimus was approved for the treatment of postmenopausal, hormone-receptor-positive metastatic breast cancer, in combination with the aromatase inhibitor exemestane. This approval was based on the BOLERO-2 study (Breast cancer trials of OraL EveROlimus-2), which demonstrated an improved median PFS for patients treated on the everolimus-exemestane arm (6.9 months), compared with exemestane alone (2.8 months). However, this PFS benefit came at the cost of increased side effects, including stomatitis (any grade: 56% vs 11%), skin rash (36% vs 6%), diarrhea (30% vs 16%), hyperglycemia (13% vs 2%), and pneumonitis (12% vs 0%). So while the approval of everolimus was an important step forward, there remained a pressing need to develop PI3K-pathway agents with an improved therapeutic index.
Consequently, there have been great hopes for direct inhibitors of the catalytic subunit of PIK3, which is encoded by the PIK3CA gene. Preclinical models of PIK3CA-driven cancers suggested that PIK3CA-mutant cell lines were considerably more sensitive to early PI3K inhibitors such as buparlisib (BKM120) and pictilisib (GDC-0941).29

Early clinical trials showed that these agents were generally well tolerated, but showed somewhat disappointing single-agent antitumor activity, even in patients with PIK3CA-mutant tumors.10,11

However, it has since become clear that different PI3K isoforms have distinct biological functions,30 and targeting these with isoform-selective PI3K inhibitors may yield an improved therapeutic index. Indeed, the early clinical trial results for α-isom form selective inhibitors of PI3K are more encouraging, and seem to have increased activity in patients with PIK3CA-mutant tumors.12,13

**FGFR inhibitors**

Mutations and amplifications in the genes encoding FGFR are among the most common abnormalities found in breast cancer. A range of FGFR inhibitors are in development, including drugs that target FGFR, but also other kinases (eg, VEGF, kinase insert domain receptor [KDR], etc), and drugs that more selectively target FGFR in isolation (Table I). While responses to these agents have been reported, they do not predictably occur in patients bearing FGFR molecular abnormalities. Current development strategies in breast cancer are focused on combination therapies with endocrine and cytotoxic chemotherapies.

**CDK4/6 inhibitors**

Disruption of the cell cycle is a well-defined hallmark of cancers in general, and many genetic alterations in cell-cycle control have been described in breast cancer specifically.4 The CDKs bind with cyclin partners to help control progression through the cell cycle, and a number of CDK-targeted drugs have been developed to interfere with this. Until recently, the clinical experience with CDK inhibitors was characterized by a lack of clinical activity and the presence of significant toxicities.31 Palbociclib was subsequently developed as a reversible, oral, small-molecule inhibitor of CDK4/6, which has an important role in regulating the G1/S phase transition via the control of retinoblastoma protein (Rb) phosphorylation. In February 2015, palbociclib was granted accelerated approval for the treatment of hormone-receptor–positive metastatic breast cancer, in combination with letrozole, based on the results from the PALOMA-1 trial (not an acronym).17 This showed a dramatic increase in median PFS from 10.2 months for patients treated with letrozole alone, to 20.2 months for patients treated with letrozole in combination with palbociclib. Confirmatory phase 3 trials are underway, and a number of other CDK4/6 inhibitors are now in clinical development.

**Immunotherapies**

Researchers have tried over many decades to enhance the patient immune response to treat cancers, unfortunately with very limited success. However, in the last few years, the development of novel immune-checkpoint inhibitors has reinvigorated the immunotherapy field. These checkpoint inhibitors block some of the inhibitory signals between effector T-cells and tumor cells, including cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed death-ligand 1 (PD-L1), thus “taking the brakes off” the immune response. Clinical trial results in melanoma and non–small-cell lung cancer have been particularly exciting, with a subset of patients achieving long-term disease control, in some cases after only a few doses of treatment.20,32 Encouraging activity has also been seen in other tumor types including bladder, head and neck, and triple-negative breast cancer. Once again, significant efforts are underway to identify biomarkers that will predict response to immunotherapies. The early data on tumor PD-L1 expression appears promising, with an enriched response rate for PD-L1–positive cancers; however, many patients with PD-L1–negative cancers also respond to therapy.21 Interestingly, the subset of breast cancer patients in the METABRIC series, which is characterized by a dearth of genomic aberrations (Cluster 4),4 is also characterized by increased immune infiltrates and may be a rational group to target with immune-checkpoint therapies. More recently, a large immunohistochemical study has revealed that approximately 20% of basal-like breast cancers express the PD-L1 protein, mostly in tumor-infiltrating immune cells.22 This subgroup could gain particular benefit from anti-PD-L1 directed therapies.

**The need for robust biomarkers to predict clinical outcome**

One common theme arching across all these clinical drug development programs is the recognition that different patients can respond very differently to any given drug. For these molecularly targeted therapies, it is imperative that robust biomarkers are identified to predict which subgroups of patients are likely to get the greatest benefit from these new drugs, which are frequently very expensive and sometimes associated with significant toxicity. There are a number of distinct categories for biomarkers correlated with drug effect; these are defined in Box 1 (page 292).10,38

**Biomarker-driven clinical trial design**

Trials investigating novel molecular therapies and biomarker profiles have required significant changes to the traditional paradigm for clinical drug development.33 In phase 1 clinical trials, the recommended phase 2 dose is now selected not only on the basis of toxicity, but is also informed by pharmacokinetic-pharmacodynamic relationships. There is now a blurring between the old division between phase 1, phase 2, and phase 3 trials, with many early phase clinical trials having both dose-escalation and large expansion phases.
Box 1. Biomarker definitions

Prognostic biomarker: prognostic biomarkers are those measured before the start of therapy in order to predict the natural history of disease. They enable patient stratification according to risk and help clinicians to treat patients accordingly. For example, patients at higher risk of tumor progression may receive more aggressive therapy. Prognostic biomarkers are also sometimes predictive (see below).

Predictive biomarker: a biomarker that is also measured before the start of therapy in order to help select which drug a patient should receive. Patients who are positive for a predictive biomarker can have a higher response rate, and those testing negative, a low response rate. Examples of predictive biomarkers in breast cancer include estrogen receptor (ER) and receptor tyrosine-protein kinase erbB-2 (HER2), and in lung cancer include epidermal growth factor receptor (EGFR) mutations and EML-ALK translocations (translocation of echinoderm microtubule-associated protein-like with anaplastic lymphoma kinase). Predictive biomarkers are also sometimes prognostic (see above).

Pharmacodynamic (PD) biomarker: a biomarker providing evidence of drug action in vivo, according to its mechanism of action. For traditional cytotoxic agents, suppression of blood counts is evidence of an antiproliferative PD effect. For modern molecular targeted therapies, PD biomarkers are often molecular readouts just downstream of the drug target. For example, evidence of the PI3 kinase inhibitor GDC-0941 hitting its target was shown by a reduction in phosphorylated AKT (pAKT) and phosphorylated ribosomal S6 kinase (pS6K) between pre- and post-treatment tumor biopsies.

Early response biomarker: an early change in this type of biomarker predicts for clinical outcome that would normally be assessed at a later timepoint. For example, a reduction in circulating tumor DNA levels in metastatic breast cancer patients in the first few weeks of treatment has been shown to correlate with a beneficial response seen on computerized tomography (CT) scan measured 2-3 months after the start of therapy.

Alongside this is a trend away from large, unselected phase 3 trials, to smaller, often phase 2 trials seeking to identify larger effect sizes in biomarker-defined patient subgroups. Furthermore, there are now major efforts in cancer centers across the world to establish routine molecular profiling services for cancer patients. These efforts help to identify groups of patients with particular molecular aberrations required for clinical trial entry, and also permit researchers to study correlations between molecular profiles and clinical outcomes (Table II).

Table II (right page). Selected clinical trial programs matching biomarker profiles to targeted therapies.

A further challenge to the basket trial, multi–tumor type approach, is that the function of particular gene mutations is often highly context dependent. For example, BRAF inhibitors work much better in BRAF-mutant melanoma than they do in BRAF-mutant colorectal cancer. Recent work from the Bernards group has suggested that this may be due to feedback activation of epidermal growth factor receptor (EGFR), which allows the cancer cells to continue proliferating in the presence of the BRAF inhibitor. This feedback loop is not seen in melanoma cells because they express low levels of EGFR.

“Umbrella” and “basket” trials

Umbrella trials tend to be multi-arm studies focused on particular tumor types. Patients are assigned to specific drugs based on the molecular profile of their cancer (Figure 2).

In a basket trial, a single drug is tested across cancer types, in patients whose tumors have been found to have a particular abnormality, often a specific gene mutation (Figure 2). One example of such a study is the VE-BASKET trial (NCT01524978);
<table>
<thead>
<tr>
<th>Trial program (Institution)</th>
<th>Tumor type</th>
<th>Archival or fresh biopsy (molecular profiling)</th>
<th>Drugs</th>
<th>Trial reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>“Basket” trial</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>VE-BASKET (Roche)</td>
<td>BRAF-mutant solid tumors</td>
<td>Archival, Fresh biopsy</td>
<td>Vemurafenib</td>
<td>NCT01524978</td>
</tr>
<tr>
<td><strong>“Umbrella” / molecular matched therapy trials: with automatic, prespecified algorithm for treatment allocation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BATTLE-1 (Houston, MD Anderson)</td>
<td>Chemorefractory NSCLC</td>
<td>Fresh biopsy (PCR, FISH): EGFR mutation / CNV, KRAS/ BRAF mutation, VEGF/VEGFR2 expression, RXRα/CCND1 expression + CNV</td>
<td>Erlotinib, Sorafenib, Vandetanib, Erlotinib + bexarotene</td>
<td>NCT00409968 (ref 39)</td>
</tr>
<tr>
<td>BATTLE-2 (Houston, MD Anderson)</td>
<td>Chemorefractory NSCLC</td>
<td>Fresh biopsy (IHC, Sequenom, RPPA, NGS Foundation Medicine)</td>
<td>Various drugs (approved)</td>
<td>NCT01248247</td>
</tr>
<tr>
<td>SHIVA (Paris, Institut Curie)</td>
<td>Solid tumors</td>
<td>Fresh biopsy (IonTorrent PGM Cytoscan)</td>
<td>Various</td>
<td>NCT01771458 (ref 40)</td>
</tr>
<tr>
<td>WINTHER (WIN consortium)</td>
<td>Solid tumors</td>
<td>Fresh biopsy: tumor and matched normal tissue (NGS, CNV, aCGH)</td>
<td>Various</td>
<td>NCT01856296 (ref 41)</td>
</tr>
<tr>
<td>FOCUS4 (Medical Research Council, UK)</td>
<td>Colorectal cancer</td>
<td>Fresh biopsies at diagnosis, on treatment and disease progression (PCR)</td>
<td>Various</td>
<td>EudraCT # 2012-005111-12</td>
</tr>
<tr>
<td>I-SPY 2 (US NCI)</td>
<td>Stage III breast cancer</td>
<td>Serial biopsies Primary resection</td>
<td>Various (adaptive)</td>
<td>NCT01042379</td>
</tr>
<tr>
<td>MATRIX (Cancer Research UK)</td>
<td>NSCLC</td>
<td>Archival, Fresh biopsy</td>
<td>Various</td>
<td>EudraCT # 2014-000814-73</td>
</tr>
<tr>
<td><strong>“Umbrella” / molecular matched therapy trials: requiring manual review of biomarker profile and treatment allocation</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SAFIR-01 (Paris, Institut Gustave Roussy)</td>
<td>Breast cancer</td>
<td>Fresh biopsy (aCGH, PCR)</td>
<td>Various</td>
<td>NCT01414933 (ref 42)</td>
</tr>
<tr>
<td>MOSCATO (Paris, Institut Gustave Roussy)</td>
<td>Solid tumors</td>
<td>Fresh biopsy (aCGH, PCR)</td>
<td>Various</td>
<td>NCT01566019</td>
</tr>
<tr>
<td>Stratified Medicine Program Phase 1 (Cancer Research UK)</td>
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<td>Archival (PCR, FISH)</td>
<td>Various</td>
<td>UKCRN 10622</td>
</tr>
<tr>
<td>IMPACT (Houston, MD Anderson)</td>
<td>Solid tumors</td>
<td>Archival, Fresh biopsy (PCR, FISH)</td>
<td>Various</td>
<td>NCT00851032</td>
</tr>
<tr>
<td>PROFILE (Boston, Dana Farber)</td>
<td>Solid tumors</td>
<td>Archival, Fresh biopsy (SNaPshot)</td>
<td>Various</td>
<td>(ref 43)</td>
</tr>
<tr>
<td>Molecular profiling service (Boston, Mass. General Hospital)</td>
<td>Solid tumors</td>
<td>Archival, Fresh biopsy (Sequenom)</td>
<td>Various</td>
<td>(ref 44)</td>
</tr>
<tr>
<td>IMPACT (New York, MSKCC)</td>
<td>Solid tumors</td>
<td>Archival, Fresh biopsy (Sequenom/HiSeq)</td>
<td>Various</td>
<td>NCT01775072</td>
</tr>
<tr>
<td>Netherlands Centre for Personalized Cancer Treatment</td>
<td>Solid tumors</td>
<td>Archival, Fresh biopsy (IonTorrent PGM SOLiD)</td>
<td>Various</td>
<td>(ref 45)</td>
</tr>
<tr>
<td>IMPACT (Toronto, Princess Margaret)</td>
<td>Solid tumors</td>
<td>Archival, Fresh biopsy (Sequenom, MiSeq)</td>
<td>Various</td>
<td>NCT01505400</td>
</tr>
<tr>
<td>Molecular profiling service (Barcelona, VHIO)</td>
<td>Solid tumors</td>
<td>Archival, Fresh biopsy (Sequenom, Illumina)</td>
<td>Various</td>
<td>(ref 46)</td>
</tr>
<tr>
<td>NCI-MATCH, NCI-MPACT (US NCI)</td>
<td>Solid tumors</td>
<td>Fresh biopsy</td>
<td>Various</td>
<td>NCT01827384</td>
</tr>
</tbody>
</table>
This work has led to combinations of BRAF and EGFR inhibitors, with chemotherapy being tested in BRAF-mutant colorectal cancer with encouraging preliminary results. Conventional clinical trial designs that aim to identify the best fit between biomarker profile and molecular targeted therapy also increasingly use multi-arm, multi-stage designs (eg, FOCUS4, [Molecular selection of therapy in metastatic colorectal cancer: a molecularly stratified randomized controlled trial program]), and Bayesian adaptive approaches (eg, BATTLE-1 [Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination], I-SPY 2 Trial [Investigation of Serial studies to Predict Your Therapeutic Response with Imaging and molecular Analysis 2]).

“Liquid biopsy” – a great new opportunity for biomarker-driven clinical drug development

This review would not be complete without considering the transformative potential of new “liquid biopsy” technologies, which provide a method to characterize tumor biomarkers over time, with repeated sampling, which is much less invasive than traditional solid tumor biopsies. At the current time, 2 of the most high-profile technologies are those to assay circulating tumor cells (CTCs) and circulating tumor DNA. In this fast-moving field, multiple research groups are investigating the potential for liquid biopsies to yield clinically useful predictive biomarkers, early-response biomarkers, and biomarkers of acquired drug resistance.

Conclusions

This review has considered how recent advances in our understanding of the molecular biology of breast cancer have led to the development of novel molecular targeted therapies. It is clear that such therapies will work best in subsets of patients defined by their biomarker profile. Novel clinical trial methodologies are being employed to help match these biomarker profiles for individual patients to the best molecular therapy, using basket, umbrella, and adaptive approaches. Current experimental therapeutics trials are translationally intensive, with repeat tumor sampling over time to capture changing molecular profiles, which occur as a result of cancer clonal evolution. These kinds of trials may be facilitated by new liquid biopsy technologies for monitoring cell-free DNA and circulating tumor cells.
Les biomarqueurs en Cancérologie

La grande variabilité de la biologie tumorale parmi les patients cancéreux détermine la réponse au traitement et les résultats cliniques. La plus grande partie de cette variabilité peut maintenant être expliquée par des différences entre les profils des biomarqueurs entre les tumeurs. Cet article analyse le cancer du sein comme axe de discussion sur les biomarqueurs actuels en Cancérologie. Les biomarqueurs les plus utilisés dans le cancer du sein sont l’expression tumorale du récepteur estrogénique (ER) et le récepteur tyrosine-protéine kinase erbB-2 (HER2), dont les hauts niveaux d’expression ouvrent la possibilité de traitements respectivement contrôlés par ER- et HER2. D’autres « pilotes » du cancer ont été identifiés ces 10 dernières années, dont certaines aberrations de la voie PI3K-AKT-mTOR (phosphatidylinositol 3-kinase ; protéine kinase B ; cible mammalienne de la rapamycine), le récepteur du facteur de croissance du fibroblaste, le contrôle du cycle cellulaire, l’angiogenèse tumorale et le contrôle immunitaire. Pour faire face à ces pilotes du cancer, un grand nombre de traitements ciblés sur de nouvelles molécules sont en développement dans le cancer du sein. Il est de plus en plus évident que la plupart des traitements ciblés sur des molécules ne fonctionneront que sur des sous-groupes de patients définis par des biomarqueurs. Ces nouveaux traitements nécessitent le développement de nouvelles méthodologies pour les études cliniques, comportant des schémas d’étude à un seul médicament (« basket ») et à plusieurs médicaments (« umbrella ») pour apprécier le traitement moléculaire au profil du biomarqueur du patient, ainsi que de nouvelles approches adaptatives bayésiennes. La clé du succès réside dans la répétition de l’échantillonnage des tumeurs des patients au fil du temps, ce qui peut être difficile en cas de biopsies invasives de tumeur, mais que les nouvelles technologies de « biopsies liquides », mesurant par prise de sang l’ADN libre circulant et les cellules tumorales circulantes, pourraient remplacer.

**Keywords:** basket/umbrella trial; biomarker profile; breast cancer; estrogen receptor, HER2, liquid biopsy; targeted therapy
Cancer burden in the world according to regional development level

by P. Grosclaude and A. Monnereau, France

Using the fifth version of GLOBOCAN software developed by the International Agency for Research on Cancer (IARC), we present the estimated number of new cancer cases and cancer deaths in the world according to level of regional development, together with time trends in incidence. There were 14.1 million new cases and 8.2 million deaths in 2012. Of the predicted 22 million new cancer cases by 2030, the greatest increases are anticipated in the low-income countries. In less developed regions, infectious agents are still the most important causes of cancer. Thus, the introduction of appropriate vaccination, early detection or screening, and effective treatment proposed to the whole population is needed. In many countries, as a consequence of the quickly evolving lifestyles and environment that go along with economic development, any reductions in infection-related cancers are offset by an increasing number of new cases that are more associated with environmental, reproductive, dietary, and hormonal factors, and possibly related to a Western lifestyle.

Medicographia. 2015;37:297-306 (see French abstract on page 306)

Cancer is a major cause of death worldwide and the first cause of death in high-income countries. It is also becoming an important cause of morbidity and mortality in less developed countries, as anticipated by the "epidemiological transition" theory. That theory on the epidemiology of population change postulates that with industrialization, the major causes of death and disability in the more advanced societies have shifted from a predominance of nutritional deficiencies and infectious diseases to those classified as chronic diseases, such as cardiovascular disease, cancer, and diabetes. Using the latest version of GLOBOCAN, we aim to describe the burden of cancer in different regions of the world according to level of development in 2012.

Materials and methods

Sources of data
We used the fifth version of GLOBOCAN (http://globocan.iarc.fr) to present the estimated number of new cancer cases and cancer deaths (thousands) and age-standardized incidence and mortality rates (per 100 000 person-years) by sex, 27 major cancer sites, and region according to development level in 2012. The designation "more developed" and "less developed" regions are intended for statistical convenience and for interpretation of trends and causes of cancer in the world. They do not necessarily express a judgment about the stage reached by a particular country.
The basic units for estimation are countries, although we present the results by level of development and for aggregated regions, as defined by the United Nations. For incidence and mortality data, the countries have been classified depending on the availability and quality of the information from population-based cancer registries (PBCR).

For incidence data, countries were rated from A to G as follows:

A. High-quality national data or high-quality regional data (coverage greater than 50% of the population),
B. High-quality regional data (coverage between 10% and 50%),
C. High-quality regional data (coverage lower than 10%),
D. National data (PBCR),
E. Regional data (PBCR),
F. Frequency data (hospital-based or pathological-based series),
G. No data.

### Incidence

| Or area in the development process (source United Nations). The complete sources and methods used in compiling cancer incidence and mortality estimates for 2012 in 184 countries worldwide can be found elsewhere. | G. No data. |

| Lip, oral cavity, and pharynx | 117.5 | 12.3 | 44.1 | 3.7 | 257.5 | 9.8 | 110.4 | 4.0 | 31% |
| Esophagus | 67.7 | 6.4 | 18.4 | 1.2 | 255.3 | 10.1 | 114.4 | 4.1 | 19% |
| Stomach | 175.1 | 15.6 | 99.4 | 6.7 | 456.2 | 18.1 | 220.9 | 7.8 | 29% |
| Colorectum | 398.9 | 36.3 | 338.0 | 23.6 | 347.4 | 13.6 | 276.3 | 9.8 | 54% |
| Liver | 92.0 | 8.6 | 42.3 | 2.7 | 462.4 | 17.8 | 185.8 | 6.6 | 17% |
| Gallbladder | 27.8 | 2.3 | 34.8 | 2.0 | 49.1 | 2.0 | 66.5 | 2.4 | 35% |
| Pancreas | 94.7 | 8.6 | 92.8 | 5.9 | 83.5 | 3.3 | 66.9 | 2.4 | 55% |
| Larynx | 50.7 | 5.1 | 7.0 | 0.6 | 87.4 | 3.5 | 11.8 | 4.0 | 37% |
| Lung | 490.3 | 44.7 | 267.9 | 19.6 | 751.3 | 30.0 | 315.2 | 11.1 | 42% |
| Melanoma of skin | 99.4 | 10.2 | 91.7 | 9.3 | 21.3 | 0.8 | 19.8 | 0.7 | 82% |
| Kaposi sarcoma | 2.6 | 0.3 | 0.7 | 0.1 | 26.4 | 0.9 | 14.5 | 0.5 | 7% |
| Breast | 793.7 | 74.1 | 88.3 | 31.3 | 90% |
| Cervix uteri | 83.1 | 9.9 | 444.5 | 15.7 | 16% |
| Corpus uteri | 167.9 | 14.7 | 151.7 | 5.5 | 53% |
| Ovary | 99.8 | 9.1 | 139.0 | 4.9 | 42% |
| Prostate | 758.7 | 69.5 | 335.0 | 14.5 | 68% |
| Testis | 32.7 | 5.2 | 22.5 | 0.7 | 59% |
| Kidney | 125.4 | 12.6 | 74.6 | 6.2 | 88.5 | 3.4 | 49.3 | 1.8 | 59% |
| Bladder | 196.1 | 16.9 | 57.8 | 3.7 | 134.3 | 5.3 | 41.6 | 1.5 | 59% |
| Brain, nervous system | 48.2 | 5.9 | 40.7 | 4.4 | 91.4 | 3.3 | 75.9 | 2.7 | 35% |
| Thyroid | 29.7 | 3.6 | 93.1 | 11.1 | 38.5 | 1.4 | 136.8 | 4.7 | 41% |
| Hodgkin lymphoma | 15.6 | 2.3 | 13.3 | 1.9 | 23.0 | 0.8 | 14.1 | 0.5 | 44% |
| Non-Hodgkin lymphoma | 101.9 | 10.3 | 88.5 | 7.1 | 115.8 | 4.3 | 79.6 | 2.8 | 49% |
| Multiple myeloma | 36.5 | 3.3 | 31.5 | 2.2 | 26.0 | 1.0 | 20.3 | 0.8 | 59% |
| Leukemia | 80.3 | 8.6 | 61.0 | 5.8 | 120.4 | 4.3 | 90.3 | 3.2 | 40% |
| All cancers excl. non-melanoma of skin | 3243.5 | 308.7 | 2832.4 | 240.6 | 4183.6 | 163.0 | 3830.6 | 135.6 | 43% |
Data from countries rated A to C were included in Cancer Incidence in Five Continents (CI5) volume IX and/or X.1,2 These countries with high-quality incidence data represent 36% (n=67) of the 184 involved in GLOBOCAN and cover most of North America, Europe, Australia, and New Zealand, and to a lesser extent, South America (6 countries including Brazil and Argentina), Asia (9 countries including Japan, India, and China) or Africa (7 countries including Algeria, Tunisia, Libya, and Egypt).1 For mortality data, countries were rated from 1 to 6 as follows:

1. High-quality complete vital registration,
2. Medium-quality complete vital registration,
3. Low-quality complete vital registration,
4. Incomplete or sample vital registration,
5. Other sources (cancer registries, verbal autopsy surveys, etc),
6. No data.

Information on trends in incidence for 6 specific cancer types (colorectal, lung, melanoma, breast, cervical, and prostate) and for several countries has been extracted from CI5plus (http://ci5.iarc.fr).4

Methods of estimation
Cancer incidence and mortality rates for 2012 by sex are estimated for the 184 countries or territories of the world having a total population greater than 200 000. Results are presented for the cancer sites or cancer types as defined by the International Classification of Diseases, Tenth Revision (ICD-10). The methods of incidence and mortality estimation are undertaken at the national level and hence their validity depends upon the representativeness and quality of the source information from the country itself (see detail in reference 1).

We identified 9 different situations with regard to data availability, leading us to use 9 specific methods of estimation of cancer incidence by country accordingly. At one end of the spectrum, when incidence data were available historically, with sufficient numbers of cases, recorded incidence rates were projected to 2012 using models and the 2012 national population applied to the fitted rates (38 countries). At the opposite end of the spectrum, when neither national or regional registries, nor national mortality data were available, and the within-country source information was either unavailable or unusable, average incidence rates from selected neighboring countries in the same region were used to derive national incidence within the country (33 countries). Between those 2

<table>
<thead>
<tr>
<th>Mortality</th>
<th>More developed regions</th>
<th>Less developed regions</th>
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<tbody>
<tr>
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<td>Female</td>
<td>Male</td>
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<tr>
<td>n ASR</td>
<td>n ASR</td>
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<tr>
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<td>15.1 1.0</td>
<td>164.2 6.3</td>
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<tr>
<td>56.1 5.2</td>
<td>15.2 0.9</td>
<td>225 9.0</td>
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<tr>
<td>106.7 9.1</td>
<td>68.0 4.2</td>
<td>362.3 14.4</td>
</tr>
<tr>
<td>175.4 14.7</td>
<td>157.7 9.3</td>
<td>198.2 7.8</td>
</tr>
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<td>80.4 7.1</td>
<td>42.6 2.5</td>
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<tr>
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<td>25.8 1.4</td>
<td>41.3 1.6</td>
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<tr>
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<td>15.0 1.2</td>
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<tr>
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<tr>
<td>197.5 14.9</td>
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</tr>
<tr>
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<tr>
<td>1591.2 138.0</td>
<td>1266.7 86.2</td>
<td>3061.9 120.1</td>
</tr>
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</table>

Table I. Estimated number of new cancer cases and cancer deaths for 27 major cancer sites by region according to level of development.

Abbreviations: ASR, age-standardized rate (per 100 000 person-years); excl, excluding; n, number of new cases (or deaths) (thousands).

situations, 7 other specific methods used the best available information on national/regional/single registries and national mortality data if available. Depending on the coverage, completeness, and degree of detail of the mortality data available, 6 methods were used, ranked in descending order of the probable accuracy of the derived estimates. In the best case, when mortality data were available historically from national sources and a sufficient number of recorded cancer deaths were available, mortality rates were projected to 2012 using models applied to the 2012 national population (69 countries). In the worst case, when recent mortality data were not available from national sources, and survival estimates could not be derived using the previous method, the country-specific rates represent those of neighboring countries in the same region (3 countries).

Trends in incidence: The CI5plus database contains updated annual incidence rates for 118 selected populations from 102 countries in the more developed regions (eg, colorectum and anus in men, lung in both sexes, melanoma of the skin in both sexes, breast, cervix, and prostate) for a specific selection of countries in the more developed regions (United States, Japan, United Kingdom, France, Italy, and Finland) compared with a selection of countries in less developed regions (Uganda, Brazil, Costa Rica, China, India, and Thailand). Figures for all cancer sites but the lung are presented with more developed countries on the left and less developed countries on the right. For lung cancer, (Figure 1) the trends are presented for females (left) and males (right). Specific comments on each cancer site will be discussed in the discussion section. For colorectal (Figure 2) and anal cancer in men, we observe downward trends in most of the more developed countries in contrast with upward trends in less developed countries, trends that are higher in emerging national economies such as Brazil and China. For lung cancer (Figure 1), we observe in more developed regions a downward trend in men and emerging upward trends (United Kingdom, France) or stabilization (United States) of the incidence in women. No specific pattern is observed in less developed regions except an upward trend in both sexes in Brazil and a downward trend for lung cancer in China (only for men). We observe positive trends in melanoma of the skin (Figure 3) in more developed regions over the study period in both sexes (ie, 1990-2007), except in Japan where incidence is low and stable. Positive trends for breast cancer (Figure 4) are observed in more developed regions—where incidence is the highest in the world—as well as in Brazil, Japan, and to a lesser extent China, Costa Rica, Uganda, and Thailand. For the cervix (Figure 5), trends are higher in less developed regions, but most of the countries selected show downward trends, except China (low stable rate) and Uganda (high positive trends). Finally, prostate trends (Figure 6) are dramatically increasing in more developed regions (with highest incidence in the black population of the United States) in contrast to lower and stable incidence rates in less developed regions, except for Brazil whose trends are similar to more developed regions.

Figure 7 (page 304) reports the number of new cancers for the 9 most frequent sites in the 6 World Health Organization (WHO) regions. Nine cancer sites represent 56.9% of all new cancers in East Mediterranean countries compared with 73.1% of all new cancers in Western Pacific countries. The case mix is relatively comparable between Europe and America, at least for the 4 leading cancer sites that represent 50% of all cancers (eg, prostate, breast, colorectum, and lung), whereas cervical, liver, and stomach cancers are more frequent in South Asia, Africa, and East Mediterranean countries.

Finally, Figure 8 (page 305) represents age-adjusted (world) incidence and mortality rates for men and women separately by region. We observed a clear contrast between more developed regions and less developed regions. Highest incidence rates in both sexes are observed in more developed regions whereas mortality rates are relatively comparable in both regions.

Discussion
Cancer is primarily a disease of older people. This observation partly explains why more developed regions bear the largest
Cancer burden in the world according to regional development level – Grosclaude and Monnereau

MEDICOGRAPHIA, Vol 37, No. 3, 2015

Lung
Age-standardized incidence rate (world); age (0-85+)

Figure 1. Trends for incidence rates of lung cancer.
Abbreviations: SEER, Surveillance, Epidemiology, and End Results Program; USA, United States of America.

Colorectum and anus
Age-standardized incidence rate (world); age (0-85+); both sexes

Figure 2. Trends for incidence rates of colorectal and anal cancer.
Abbreviations: SEER, Surveillance, Epidemiology, and End Results Program; USA, United States of America.

Melanoma
Age-standardized incidence rate (world); age (0-85+)

![Melanoma Incidence Rates](image)

**Figure 3.** Trends for incidence rates of melanoma.
Abbreviations: SEER, Surveillance, Epidemiology, and End Results Program; USA, United States of America.

Breast
Age-standardized incidence rate (world); age (0-85+)

![Breast Incidence Rates](image)

**Figure 4.** Trends for incidence rates of breast cancer in females.
Abbreviations: SEER, Surveillance, Epidemiology, and End Results Program; USA, United States of America.

Cervix
Age-standardized incidence rate (world); age (0-85+)

Figure 5. Trends for incidence rates of cervical cancer.
Abbreviations: SEER, Surveillance, Epidemiology, and End Results Program; USA, United States of America.

Prostate
Age-standardized incidence rate (world); age (0-85+)

Figure 6. Trends for incidence rates of prostate cancer.
Abbreviations: SEER, Surveillance, Epidemiology, and End Results Program; USA, United States of America.

proportion of the cancer burden: 43% of the global incidence and 35% of the global mortality is due to cancer in a population representing 18% of the world’s population. Obviously, the populations of more developed regions are older than in less developed regions, but our interpretation of the burden of cancer in the world needs to be done in light of the knowledge of cancer as a heterogeneous mix of diseases. Every cancer has specific risk factors that will influence the trends in incidence and a wide spectrum of prognosis that will have an impact on mortality rates. The availability of effective treatments and the access to innovative drugs in each country are also crucial to the interpretation of the mortality rates and the trends.

In more developed regions, the upward trends in incidence are essentially influenced by incidence in 2 cancer sites: breast in women and prostate in men. These results are interpreted to be related to demographics and risk factors, but are mostly due to the dissemination of early detection and screening procedures. A high level of participation in screening artificially increases the cancer incidence in the population, by increasing the numbers of detected cancers that are small in size and have a good prognosis. Prostate and breast cancer, as well as thyroid cancer, illustrate this situation. However, though screening procedures usually tend to cause an increase in cancer incidence, the impact may be rather low when the screening target is a precursor lesion of cancer, such as in cervical cancer. Indeed, screening is effective for cervical cancer, and its impact on incidence is showing a clear decrease, owing to the treatment of the precursor lesion before the appearance of the cancer. Lung cancer is the most frequent cancer site worldwide. It is the leading cause of cancer deaths in men in many countries, and is the most common form of cancer death in women in North America, Northern Europe, and China. The incidence rates observed around the world are closely related to tobacco smoking, as shown by

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**Figure 7.** Number of new cancers for the nine most frequent cancer sites in the six World Health Organization regions.

the trends and the geographical distribution of the prevalence of tobacco consumption worldwide. The impact of atmospheric pollution comprising air pollution inside houses (cooking or heating) or outside (industry and transport) differ from one country to another, but should not be overlooked. Incidence rates for lung cancer are always higher in men than in women, whereas downward time trends are observed in men in numerous countries, contrasting with upward time trends in women. Lung cancer and other tobacco-associated cancers, although not placed among the leading cancers throughout less developed regions at present, will become a serious problem unless tobacco smoking is effectively controlled.

Data published recently by Bray et al using the GLOBOCAN database on year-of-incidence 2008, reported that in more developed regions (ie, having a high Human Development Index [HDI]), breast, lung, colorectal, and prostate cancer accounted for half of the overall cancer burden, whereas in medium-HDI regions, cancers of other sites such as the esophagus, stomach, and liver were also common. In contrast, female breast and liver cancers were common in less developed areas, together with 2 additional infection-related cancers such as cervical cancer and Kaposi sarcoma in some African countries.

In 2008, the attributable fraction for infectious agents was 22.9% in less developed countries and 7.4% in more developed countries. Hepatitis B and C viruses, human papillomaviruses, and Helicobacter pylori were responsible for about 2 million cases, mainly liver, cervical, and gastric cancers. Application of existing public health methods for infection prevention, such as vaccination, safer injection practice, or antimicrobial treatments, could have a substantial effect on the future burden of cancer worldwide.

If the cancer- and sex-specific trends estimated using GLOBOCAN continue in the same way, predictions show that one can expect more than 7 million new cases worldwide between 2008 and 2030 (ie, 14 million new cases in 2008 to 21.6 million by 2030). Of the 7 million new cases, 5 million will occur in less developed regions (ie, 8 million new cases in 2008 to 13 million by 2030). Cancer burden in those areas will probably become a more serious problem and one of a different nature in the future than at present.

In many countries, decreases in cervical and stomach cancer incidence seem to be offset by increases in the incidence of colorectal cancer, breast cancer in females, and prostate cancer in males. Besides the effect of screening or early detection, which we evoked previously, it is necessary to take into account the increase of well-known risk factors. The fast evolution of lifestyles and environment, which goes hand in hand with economic development in many countries, offsets any reductions in infection-related cancers by an increasing number of new cases that are more associated with environmental, reproductive, dietary, and hormonal factors, and possibly related to a Western lifestyle.

By analogy with the concept of epidemiological transition, we can speak about epidemiological cancer transition. We observe a shift from infection-related cancer predominance to cancers associated with risk factors that are mainly noninfectious and possibly related to environmental factors, a situation that arises in less developed regions.
Conclusion
Recent trends in cancer incidence in more developed areas may allow us to anticipate the longer-term evolutions in the rest of the world. Some evidence shows the simultaneous start of a decline in lung or oral cavity cancer incidence, at least in men, and the rise and increasing relative importance of other cancer types (eg, pancreas, ovary, testis, kidney, or lymphoma). For these cancers, the causes are often unknown or explain only a small part of the incidence and effective treatments are not always available. A shift to “new priorities” for developed countries is apparent: on one hand, better understanding the origin of these cancers and development of more effective treatments and on the other hand, avoiding overtreatment and overdiagnosis. However, it is necessary to maintain or strengthen primary prevention. It is increasingly obvious that global cancer prevention requires an integrated approach to the prevention of other chronic diseases, including heart disease, stroke, diabetes, and chronic respiratory diseases, as these share major underlying risk factors.

Of the predicted 22 million new cancer cases by 2030, the greatest increases are anticipated in the low-income countries and longer-term planning is needed to reduce the future cancer burden through resource-appropriate interventions. In less developed regions, infectious agents are still the most important causes of cancer. Thus, the introduction of appropriate vaccination, early detection or screening, and effective treatment proposed to the whole population is needed. This includes interventions targeting lifestyle factors amenable to reduction through proven actions. Primary prevention strategies must be developed, such as tobacco avoidance or cessation, reduction in alcohol consumption, combat of unhealthy diets and physical inactivity. Finally, the fight against cancer cannot rely solely on people to change their behaviors. It requires a comprehensive and integrated approach that includes legislation and regulation that modify environmental factors. Furthermore, programs for cancer care tailored to the needs of individual low-income countries need to be developed and tested.

References

Keywords: cancer burden; epidemiological transition; GLOBOCAN; incidence rate; mortality rate; regional development level; trend

POIDS DU CANCER DANS LE MONDE SELON LE NIVEAU DE DÉVELOPPEMENT RÉGIONAL
Nous présentons le nombre estimé de nouveaux cas de cancer et de morts par cancer dans le monde selon le niveau de développement régional et avec les tendances temporelles de l’incidence à l’aide de la 5e version du logiciel GLOBOCAN développé par l’IARC (International Agency for Research on Cancer). En 2012 il y a eu 14,1 millions de nouveaux cas et 8,2 millions de décès. Sur les 22 millions de nouveaux cas de cancer prévus pour 2030, les augmentations les plus importantes sont attendues dans les pays à faibles revenus. Dans les régions moins développées, les agents infectieux sont encore les causes les plus importantes de cancer. Il faut donc mettre en place pour l’ensemble de la population, des vaccinations appropriées, une détéction ou un dépistage précoces et des traitements efficaces. Dans beaucoup de pays, toute diminution des cancers liés aux infections est contre-balanclée par une augmentation de nouveaux cas de cancer plus associés aux facteurs environnementaux, de reproduction, alimen
taires et hormonaux peut-être liés au mode de vie occidental, comme une conséquence de l’évolution rapide des modes de vie et de l’environnement, parallèle au développement économique.
THE QUESTION

Traditional chemotherapy remains an important mainstay of cancer treatment, in spite of its toxicity for “innocent bystander” cells. Leaps in understanding of cancer biology have fostered growing excitement toward a more precise, individualized approach using targeted therapies. Nevertheless, teasing out the vulnerabilities of an individual patient’s cancer and targeting those successfully is still an emerging science. With some remarkable successes under our belt, how close are we to using such an approach for every patient?

Will all cancer patients be treated by targeted therapies in the next 10 years?

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3. W. Janni, Germany
4. B. Jeziersek Novakovic, Slovenia
5. W. S. Kim, Republic of Korea
6. A. A. Kovalev, Ukraine
7. M. Krzakowski, Poland
8. A. Rosta, Hungary
9. P. Saip, Turkey
10. M. Simonelli and A. Santoro, Italy
11. J. Tabernero, Spain
Our understanding of the mechanisms underlying development and progression of any disease fundamentally influences treatment choices. In cancer, unprecedented technical advances in recent years have furthered our understanding of the disease, fostering the emergence of concepts such as targeted therapies and personalized or individualized medicine.

While we have witnessed significant improvements in the management of certain groups of patients with the introduction of monoclonal antibodies and tyrosine kinase inhibitors, the war on cancer is certainly far from over.

The clinical benefit we have observed in a limited number of patients has given rise to an expectation that sequencing the genome of cancers would result in the identification of targetable driver genetic abnormalities in all cancers. However, the effort to unveil the genetic makeup of most tumors has made evident something that we inherently knew all along: cancer is a heterogeneous, diverse, complex, and biologically very resourceful disease. It would be naive to believe that our current understanding holds the key that will unlock its true secrets. The complexity and heterogeneity we have come to recognize, while not inconsistent with our successes with applying the targeted approach, with fantastic benefits in tumors that have a simpler or much more primitive biology (single driver or overdependence on a single pathway), they are certainly consistent with our failures.

Indeed, development of resistance has been almost universal in common tumor types with complex genomes after an initial response to a targeted agent. We are only beginning the process of properly studying and understanding resistance, something essential to the rational design of new treatment approaches and to the tailoring of combination strategies. To add to the complexity, recent evidence again points to the microenvironment, after our first encounter with clinical antiangiogenesis approximately 15 years ago. The immune system, with its yet incompletely understood complexity, has been considered the target of the year, raising the hopes of patients and treating physicians. Published results allow us to be cautiously optimistic, but in reality not all patients do benefit and much more work lies ahead in this field.

As we face this challenging disease in the future, the true “magic bullet” will likely be in the form of a rational combination of approaches. In other words, using all the potentially effective treatments we have at hand in the best and most effective regimen and in the ideal sequence, an elusive concept in the current treatment guidelines for most tumor types. I do believe that in the next 10 years, patients with cancer will continue to be treated with a combination of approaches. This includes a more rational indication of surgical procedures, both for primary tumors and with an expanding use in metastatic lesions. Radiotherapy will follow the same path with an increased use in localized lesions, particularly in oligometastatic situations with slow progressive disease.

Chemotherapy is not obsolete, yet. It will be important in combination regimens and will remain an alternative for patients with progressing disease on targeted treatments.

The combination of targeted agents directed both at tumor abnormalities and at emergent microenvironment targets seems to represent the most elegant and promising approach to focus on in clinical experimentation.

Looking back, too often we have optimistically embraced novel approaches as if a Holy Grail that would save our patients from their terrible tumors. We have as often ended up shamefully recognizing that the complexity of cancer requires a more cautious and humble attitude. With this in mind, we can say that targeted therapies are here to stay, and will continue to benefit not all, but a significant number of patients with different tumor types. However, we should also expect a more complete understanding of cancer biology to guide us toward an ever more rational treatment approach.
For decades, the hallmark medical treatment for cancer has been intravenous cytotoxic chemotherapy. Such drugs target rapidly dividing cells. This includes the intended target—cancer cells—but also certain normal tissues, such as the rapidly dividing cells of gastrointestinal tract mucosa, hair follicles, and bone marrow which are usually caught in the cross fire, hence the common toxicities of chemotherapy. Today, with a better understanding of the disease, we know of other targets, other mechanisms for dealing with tumor cells, and other drivers accelerating or decelerating tumor growth. We also know how tumors steal their blood supply from surrounding tissues, and we try to stop that process.

We have begun to realize how tumor cells differ from normal cells, and we target these differences to achieve the maximum effect of therapy with the minimum level of side effects.

For now, chemotherapy remains the main weapon available, but there is a rapidly developing role for new, targeted therapies. These are becoming an essential component of any modern therapy for various tumors.

Targeted therapy is not especially new. In breast cancer, steroid receptors—determined to be drivers of cell proliferation in that disease—were perhaps for the first time used to characterize and target cancerous cells, with a dramatic response of steroid-receptor–positive cancer to hormonal manipulations with antiestrogens.3

Ever since, our thinking on how to deal with tumors has changed. Now we aim to steer therapeutic strategies away from the use of chemotherapeutics as weapons of mass destruction and toward the use of targeted or smart bombs.

A number of targets have already been identified. One example is the epidermal growth factor receptor (EGFR) found on the surface of tumor cells, which we target with antibodies to reverse the devastating proliferative effects. There is evidence that it is beneficial to combine those antibodies with chemotherapy in the management of various solid tumors.2

The birth of trastuzumab was a revolution in breast cancer therapy, with a doubling in survival in patients with tumor cells bearing an excessive number of copies of receptor tyrosine-protein kinase erbB2 (ERBB2 [formerly HER2 or HER2/neu]) on their surface.1,4 Contrary to almost all chemotherapeutics, which need rapid division of DNA to exert an action, the new targeted therapy acts on dividing and nondividing cells.

Additionally, the discovery that activation of membranous receptors leads to signal transduction, a series of activation of intracellular proteins propagating signals to the nucleus, increasing the rate of division and proliferation, led to another category of targeted therapies, the tyrosine kinase inhibitors. Examples include sunitinib for renal cell carcinoma5 and sorafenib for hepatocellular carcinomas, which have the advantage of being orally delivered.6 Furthermore, antiangiogenic agents have created quite a stir. Such agents use antibodies to target the factor that promotes angiogenesis, vascular endothelial growth factor (VEGF), with the goal to stop cancers from gaining a blood supply. The antiangiogenic bevacizumab is a good example, with a great response in brain gliomas and colon, breast, and ovarian cancers.7-10

As our understanding grows, we are better able to specifically target tumor cells, sparing normal cells. With many more discoveries expected, I am convinced that targeted therapies are the future of cancer treatment and that the next 10 years will see considerable progress in that regard.

REFERENCES
Two targeted therapies are currently widely used in breast cancer patients: endocrine treatment for hormone-receptor–positive breast cancer and, since the 1980s, receptor tyrosine-protein kinase erbB2 (ERBB2 [formerly HER2 or HER2/neu])-targeted treatment in patients with overexpression of the ERBB2 receptor or amplification of the ERBB2 gene. Endocrine treatment, the oldest form of targeted treatment in the breast, has convincingly demonstrated its effectiveness for more than 5 decades. While practically all patients with hormone-receptor–positive breast cancer receive endocrine therapy at some point in their disease—in most cases, during the vast majority of the active treatment time—the development of ERBB2-targeted treatment impressively demonstrates the potential of other targeted therapies in breast cancer.

For many years, trastuzumab was the only treatment option in ERBB2-overexpressing advanced breast cancer. In 2007, lapatinib was approved by the US Food and Drug Administration in combination with capcitabine, and in 2010, with an aromatase inhibitor. A meta-analysis has shown a significant benefit in progression-free survival (PFS) and overall survival (OS) with a lapatinib-containing regimen in patients with locally advanced or metastatic breast cancer (MBC). Increasing amounts of data suggest that dual-targeted treatment, combining either trastuzumab with lapatinib or with pertuzumab might significantly increase efficacy of ERBB2-targeted treatment in a clinically relevant setting. In the metastatic setting, the CLEOPATRA study (Clinical Evaluation Of Pertuzumab And TRAStuzumab) randomized 808 ERBB2-positive patients to receive placebo plus trastuzumab plus docetaxel, or pertuzumab plus trastuzumab plus docetaxel as first-line treatment until disease progression. Results showed a significant increase in median PFS in the pertuzumab group. In a recent second interim analysis, a significant OS benefit was shown for the dual blockade treatment. A phase 3 study that compared lapatinib monotherapy with a combination of lapatinib and trastuzumab in heavily pretreated patients showed a significant OS benefit with dual-targeted treatment.

In analogy to endocrine treatment, the continuation of targeted treatment in ERBB2-positive patients with progressive disease on previous ERBB2-directed therapy has been well established. Four retrospective cohort studies, as well as prospective randomized controlled trials, demonstrated a significant benefit in PFS and OS when ERBB2-targeted treatment was applied beyond disease progression, in combination with alternative cytostatic or endocrine agents. In a recent pooled analysis comprising the data of 2618 patients, Peteelli et al. calculated a benefit in (weighted median) OS of 24 months in patients who received continued trastuzumab beyond disease progression.

While targeted treatment in patients with hormone-receptor–positive and/or ERBB2-overexpressing breast cancer has become standard care, targeted treatment for triple-negative breast cancer is currently not available. However, even for this entity, novel agents, such as a group of pharmacological inhibitors of the enzyme poly(ADP ribose) polymerase (PARP inhibitors), based on homologous recombination deficiency, are under development and have demonstrated superior efficacy in combination with platin-based chemotherapy compared with chemotherapy alone.

These early data and the existence of an array of other novel biological agents show promise that the gap for targeted therapy will close, even for the approximately 15% of breast cancer patients for whom no targeted treatment is currently available. Therefore it is reasonable to speculate that targeted therapy will be used in almost all breast cancer patients within the next 10 years, reducing even further the mortality of that disease.

References
Enormous advances in molecular biology have helped identify specific molecular abnormalities that characterize a certain type of cancer in a unique manner, thus allowing the development of specialized molecules that can selectively target the specific cancer cell population and minimize the hazardous effects on normal cells. By definition, molecularly targeted therapy refers to every specific treatment strategy directed against well-defined targets characteristic for cancer cells and therefore includes various categories of concepts: the inhibition of receptor function, inhibition of cytoplasmic signal transduction, inhibition of the cell cycle, modulation of apoptosis, antiangiogenic and antivascular activity, anti-inflammatory activity, interaction with the telomeres and telomerase activity, as well as transversal mechanisms (eg, histone deacetylase inhibition, proteasome inhibition, heat shock protein inhibition). At the beginning of December 2014, more than 57 targeted therapies were approved by the US Food and Drug Administration for 77 malignant diagnoses and the list of these therapies is continuously expanding.

However, regardless of improved cancer detection through development of new technologies and biomarkers as well as cancer prevention programs and despite superior local therapies, there will still remain quite a population of patients in need of an effective systemic treatment. There are 2 equally important scenarios for an adequate consideration of these patients—the first being the reactivation of immune system mechanisms in patients having a smaller tumor burden following successful local therapies. With this scenario, the activated immune mechanisms will be capable of a competent surveillance of the remaining cancer cells, resulting in their elimination. The treatment modality of this kind primarily includes different kinds of tumor vaccines, which are, in general, supposed to be capable of restoring the effectiveness of immune defense, either by elimination of immune suppression or by enhancing the activities of immune effector cells. In patients in whom the extent of malignant disease is beyond the control of the immune system, there will be a need for the second scenario, ie, effective targeted therapies with a direct blocking effect on the growth and spread of cancer cells.

The prerequisite for such treatments will, however, be a profound and accurate knowledge of exact mechanisms of specific cancer development and its behavior. In addition to primary targeted therapies targeting the most common specific abnormality in the cancer cell, there will be a need for the development and application of secondary targeted therapies. These therapies are supposed to affect the less common targets in the same or different signaling pathways with the intent to prevent the development of resistance to primary targeted therapies and to improve their efficacy.

In the next 10 years, systemic treatment of cancer will experience further expansion, a more modest one in the field of conventional therapies and an exponential one in the field of second- and maybe even third-line molecularly targeted therapies. Owing to the substantial effectiveness of conventional therapies in some cancers, eg, germinal cancers and lymphomas, this kind of treatment will remain the cornerstone of treatment for those types of cancer. On the other hand, targeted therapies will predominate in the treatment of other types of cancer, either alone, in combination with conventional therapies, or in combination with other second-line targeted therapies.

References
We have long aimed to cure cancer. Most simple approaches include removal or destruction of cancer cells by surgical excision, radiation, or use of toxic chemicals, so-called chemotherapeutic agents. Though achieving a level of success with those treatment strategies, it has been less than satisfactory.

Looking to the future, our growing knowledge of cancer cell biology is leading to better treatment. One example of a treatment based on cancer cell biology is antiandrogen therapy in prostate cancer. In most cases, simple orchiectomy or hormonal modification can suppress tumor growth. Another example is all-trans retinoic acid (ATRA) for acute promyelocytic leukemia. This previously fatal leukemia is now considered one of the favorable acute leukemias. However, finding a more powerful and specific biologic target remains a challenge.

Prior to the era of genomics, we had already identified some biologic targets. More than 90% of chronic myelocytic leukemias (CML) have a specific chromosomal translocation, previously known as the Philadelphia chromosome. The specific genes located in this fusion are ABL from chromosome 9 and BCR from chromosome 22. The BCR-ABL fusion protein makes ABL tyrosine kinase constitutively hyperactive, resulting in inappropriate proliferation of hematopoietic cells. Therefore, the BCR-ABL fusion protein is an obvious therapeutic target. Only 1 drug—inatinib—can change the entire natural history of CML, which it does by inhibiting the tyrosine kinase activity of the BCR-ABL fusion protein. That success is proof of the potential of targeted therapy.

How much do we understand about carcinogenesis? How can we get information on the pathogenesis of each cancer type? Nowadays, treatment strategies for the management of cancer are rapidly transitioning. Very common key words we come across in recent reports include “targeted,” “genomic,” and “personalized.” How can we make these changes obtainable? For the last decade, genome-wide analysis has been in development, owing to the considerably reduced cost of next-generation sequencing and to improved performance in deciphering genomic information. This genomic knowledge may lead to optimal targets. Apart from targeting intracellular pathways, monoclonal antibodies can be considered another part of targeted therapy. The idea of “magic bullets” in medical treatment, a kind of chemotherapy, emerged in the late 19th century. However, it would take more than 100 years for the idea to come to fruition in cancer therapy, with monoclonal antibody–based treatment having been established since the year 2000. Why did it take so long? The idea could not be realized before monoclonal antibody technology and capability for modification were in place. Nowadays, monoclonal antibodies are an essential part of cancer treatment.

Nevertheless, considering whether targeted therapy will replace traditional chemotherapy in the next decade, I admit I do not believe that will happen, not totally. First of all, we have made great success in treating some cancers with very inexpensive chemotherapeutic agents alone. For example, consider how the natural history of Hodgkin lymphoma has changed from 50 years ago. That success is attributable to the combination of inexpensive and long-known chemicals. Second of all, targeted therapies are too expensive. In most underdeveloped countries, health care resources are limited, and there is a great burden on such resources in developed countries as well. Thus, even looking 10 years into the future, chemotherapeutic agents can continue to be powerful tools in cancer treatment. Targeted agents may increasingly replace chemotherapeutic agents to reduce toxicities and enhance efficacy; however, it will be difficult to treat all cancer patients with targeted agents within the next decade.
With cytotoxic cancer chemotherapy reaching the limits of its potential by the late 1980s, the treatment paradigm for cancer required a change. Advances in molecular biology and a focus on personalized medicine led to a different approach, using new, molecularly targeted drugs. Today, blockade of cancer cell proliferation is achieved through selective inhibition of the main pathways driving that process.

The first and most impressive examples of the new, targeted approach were the use of imatinib in chronic myelocytic leukemia and gefitinib in lung adenocarcinoma, their effectiveness dependent on gene variants of individual tumors.

In the last decade, personalized targeted cancer therapy was shown to be effective in tumors of different localizations (breast, lung, colorectal, stomach, and kidney cancers, melanoma, and others). There is, however, an urgent need to review the treatment paradigm, as there have been many clinical failures due to primary and acquired tumor resistance.

The insufficient effectiveness of molecularly targeted drugs is not due solely to genetic heterogeneity and clonal evolution of cancer cells. Indeed, there are other contributing complexities related to the permanent biological variability of all components involved in tumor progression (cells of primary tumors and components of the metastatic cascade).

Tumor resistance mechanisms toward different targeted drugs are well known. They are related to the activation of alternative epidermal growth factor receptor (EGFR) pathways, enabling the cell to survive in response to drug-induced damage, as well as formation of an oncogenic bypass and autocrine loop, loss of the extracellular domains of membrane receptors (truncation), kinome reprogramming, autophagia, epithelial-mesenchymal transition, epigenetic mechanisms, etc. During disease progression and upon influence of therapy, additional oncogenic mutations can emerge and the molecular landscape change. This is known as genomic chaos.1

We have now come to realize that cancer cell development is possible only in microenvironments consisting of benign stromal cells, immune cells, and inflammatory cells interacting with one another in complex synergistic, antagonistic, and mutual interrelations, with the evolution of a malignant clone resulting. Two classic papers of Hanahan and Weinberg2,3 present the most comprehensive view of cancer evolution. Based on presented features, targeted therapy should not focus only on cancer cells with their instable genome; special form of metabolism; active neoangiogenesis; and acquired ability to avoid signals for growth, to circulate in the blood, and to metastasize. Other targets, such as the tumor microenvironment, cancer stem cells, and all components of the metastatic cascade should be considered. Nevertheless, implementing such a program as a treatment protocol for individual patients would be impossible, even using a combination of several targeted drugs. Furthermore, a single drug with a unique molecular mechanism of action could not be considered an adequate treatment for progressing genetically heterogeneous tumors.

The insufficient effectiveness of therapy in cancer is due mainly to the complex biology of the disease and the lack of new scientific explanations of the early mechanisms of cancerogenesis and tumor progression. Indeed, Lineweaver et al4 suggests that cancer is an ancient evolutionary survival tool in multicellular organisms. Thus, we are trying to overcome an extremely complex evolutionary process that’s been going on since the Metazoan period and over the last billion years.

Advances in cancer therapy in the next decade will most probably involve new potential to influence a patient’s immune system (the process of immunoediting) as well as pharmacological influences on energetic metabolism in cancer cells (metabolic reprogramming). The literature suggests that the most impressive advances in recent years were achieved along those lines. Targeted antitumor drugs will not be abandoned to oncology history within the next 10 years. Rather, they will be integrated into the general paradigm of cancer therapy, which should continue to steadily change as scientific data emerges and is reexamined.

References
Over the past decade, new genomic and proteomic technologies enabled the acquisition of a plethora of information fundamental to the understanding of the nature of cancer. Genetic and molecular characteristics are increasingly relied upon in clinical practice: they may be essential to planning optimal screening, for correct diagnosis, for providing a reliable prognosis, and for selecting the best treatment. It is important to explore genetic and molecular changes in cancer and to incorporate this information into clinical practice, following good examples of initiatives like The Cancer Genome Atlas. Genetic and molecular analyses may speed up the identification of various subtypes in different cancers and the development of new anticancer agents. An example is renal-cell carcinoma. Identification of molecular changes underpinning its growth accelerated development of new antiangiogenic therapies (eg, multikinase inhibitors and other drugs).

What are today’s demands in the field of targeted anticancer therapy? Will all patients with cancer receive molecularly targeted treatment in the next years? The answers are not simple and several aspects should be taken into consideration. Taking a look at the current needs in targeted anticancer therapy, more investment in the development of genomic and proteomic research is required, investments not limited to obtaining financial resources, but aiming as well to organize international platforms or programs for biomarker qualification and validation. This is of outstanding importance, not only for frequently diagnosed neoplasms (eg, lung cancer or colorectal cancer), but mandatory in so-called rare malignancies (eg, malignant pleural mesothelioma, gastrointestinal neuroendocrine neoplasms).

Additionally, we should stimulate genetic and molecular research aiming to identify resistance mechanisms. A very good example is non–small cell cancer (adenocarcinoma in particular) and the classification algorithm based on molecular findings: success of anti-epidermal growth factor receptor (EGFR) small-molecule inhibitors should be followed by research on mechanisms of resistance (eg, T790M mutations) and methods to overcome the problem (eg, new anti-EGFR agents).

Furthermore, it is necessary to focus research on biomarkers of toxicity. Targeted agents are not free of adverse side effects; however, such effects are frequently different to those seen with conventional cytotoxic agents. Unfortunately, very little is being done to identify biomarkers that are useful for predicting treatment-related toxicity. Similarly, the search for biomarkers should also identify factors that may predict response to cytotoxic agents.

There is also a need to remove all barriers and obstacles to patient participation in clinical trials that address targeted agents. Such barriers exist, at least in some countries. Central coverage of routine medical costs for patients participating in approved research studies should be introduced in many countries (eg, Poland).

Finally, it is important to address pricing of targeted agents, ensuring they are sensible and affordable in countries with a limited budget.

Considering whether all cancer patients will receive molecularly targeted therapies in the next decade, I do believe such medical strategies (in oncology in particular) represent the future. The history of hormonal receptors and antiestrogen agents—the archetype of targeted therapy—serves as a lesson. When tamoxifen was introduced, a small proportion of patients with breast cancer received this agent; nowadays, approximately 80% of breast cancer patients receive adjuvant hormonal treatment. Furthermore, molecularly targeted treatment with imatinib dramatically changed the management of gastrointestinal stromal tumors. Non–small cell lung cancer patients with advanced disease are now given therapy that is directed toward specific aberrations within the EGFR or ALK genes, rather than suffering unnecessarily from adverse side effects of chemotherapy.

Nevertheless, I do not think that all patients will receive targeted agents within a 10-year perspective. The role of conventional chemotherapy and other treatment modalities will be preserved, but patients will be given targeted treatments when truly justified. In other words, we are approaching the time of “appropriate drug for the individual patient,” meaning optimal effectiveness and safety.
Molecularly targeted cancer therapy targets a specific, predefined molecule and the signaling pathway it oncogenically transforms. Such therapies use diverse tumor biological approaches, including, but not limited to, hormone and hormone receptor–antagonist therapies of hormone-sensitive tumors, inhibition of cell surface growth factor receptors, activation of antitumor T-cell immune responses, chimeric antigen receptor (CAR) T-cell therapy, monoclonal antibodies conjugated with an effective toxin or radioactive isotope, and unconjugated monoclonal antibodies.

The above-mentioned new cancer therapies were developed to offer more effective treatment modalities that selectively target tumor cells, having fewer adverse side effects than conventional cancer therapies. Although routinely used targeted therapies have resulted in significant progress in the treatment of certain tumor types like chronic myelocytic leukemia and gastrointestinal stromal tumors with the BCR-ABL gene product inhibitor imatinib, as well as the second- and third-generation BCR-ABL kinase inhibitors, no such remarkable success has been observed in any of the solid tumors.

Genomic, proteomic, and epigenetic research reveal an increasing number of molecules that play a key role in the oncogenic transformation of signaling pathways and that are or will be targeted in the future. However, there are some general issues with targeted cancer therapies in solid tumors.

The first issue is efficacy. Many of the targeted therapies show clinically relevant efficacy in solid tumors, but the duration of response varies and in many cases is relatively short (only a few months), with considerable toxicity and rather significant costs. In solid tumors, the efficacy of targeted therapies is significantly decreased by intratumor heterogeneity, which is a well-known tumor-biology phenomenon. The duration of response of an initially effective targeted therapy depends on the degree of this heterogeneity, also called molecular diversity. The reason for resistance is the developing dominance of tumor cells carrying the mutant target molecule or the activated bypass signaling pathways. This problem is further complicated by the fact that tumor diversity and heterogeneity increase during cancer progression, and the molecular diversity of primary tumors and metastases differs both quantitatively and qualitatively.

The next issue is toxicity. The aim of the targeted-therapeutic principle was to develop more-effective cancer therapies with more-favorable toxicity profiles and fewer side effects compared with traditional cancer therapies. However, targeted therapies also have several potentially life-threatening side effects. The adverse-event profiles differ from conventional cancer therapies, but also necessitate supportive care and this requires funding sources. The accelerated approval process of targeted cancer therapies also poses a problem. The few clinical studies performed up to the time of approval does not provide a complete picture of toxicity, therefore pharmacovigilance is of critical significance during use of these therapies in clinical practice.

The third and main issue is cost, which, objectionably high, makes treatment inaccessible to most patients. Currently, only 10% of patients have access to the effective targeted cancer therapies, an unacceptable rate. While partly explained by high costs of research and development, inadequate pricing and profit expectations of manufacturers probably play a role. The degree of efficacy, duration of response, and the realistic cost/benefit calculations of a given targeted cancer therapy will be essential for assessing the reimbursement amount provided by government health care programs.

Research results will help reveal mechanisms of resistance and allow increasing efficacy with the use of combined or sequentially applied targeted treatment regimens, avoiding some of the resistance mechanisms, and prolonging duration of the therapeutic response. However, in my opinion, targeted therapies will not predominate in the treatment of solid tumors in the near future, but will be used as part of a comprehensive cancer therapy approach in an increasing number of tumor types in any line of treatment.

References
Significant advances have been made in the treatment of most cancers with the development of targeted biological agents. Agents approved for use against specific cancers include those that prevent cell-growth signaling, interfere with tumor blood vessel development, stimulate the immune system to destroy cancer cells, and deliver toxic drugs to cancer cells. Although real improvements have been shown in the treatment of tumors—such as estrogen receptor (ER)-positive breast cancer, receptor tyrosine-protein kinase erbB2 (ERBB2 [formerly HER2 or HER2/neu])-positive breast and stomach cancer, c-kit–positive gastrointestinal stromal tumors, BCR-ABL fusion protein–positive leukemia, serine/threonine–protein kinase B-Raf (BRAF)-positive melanoma, and epidermal growth factor receptor (EGFR)-positive adenocarcinoma of the lung—advances in most of the cancers are modest. Because the targeted therapies are often cytostatic, the ones that really cure cancer are rare.

The transition from cytotoxic to targeted therapeutics occurred with the improved understanding of tumor biology and with the technological advances in molecular sequencing of the human genome and genomic profiling of different tumor types. However, not all successes at the bench in preclinical experiments have translated to success at the bedside, at least not yet. Sometimes a genetic abnormality may be important for a specific drug in a specific cancer, but targeting the same genetic abnormality in a different cancer does not work. For example, the lack of correlation between EGFR overexpression and response to EGFR inhibitors showed the importance of evaluating patient selection biomarkers in the preclinical setting before clinical implementation. The main obstacles to success are intratumoral and intertumoral heterogeneity, therapeutic resistance, and tumor microenvironment. For some types of cancer, most patients with that cancer have an appropriate target for a particular targeted therapy and thus are candidates for that therapy. For example, in chronic myelocytic leukemia (CML), most patients have the BCR-ABL fusion gene. In contrast, Ras is mutated in 25% of all cancers, but it has not been possible to develop inhibitors of Ras signaling.

The most important limitation of targeted therapy at present is that drugs for some identified targets are difficult to develop because of the target’s structure and the way its function is regulated in the cell. Also, we are still using targeted agents like angiogenesis and mammalian target of rapamycin (mTOR) inhibitors, with no real targets within the tumor itself. If these obstacles can be overcome, guiding the choice of molecularly targeted treatment for most cancers can be a reality in the next 10 years.

Next-generation sequencing, which is very important for the application of targeted therapies, is evolving, but not yet ready for standard use. We do not know how accurate these assays are and they sometimes detect genetic abnormalities that may not have biologic and clinical significance. Progress in molecular oncology has led to rapid adjustments in the design of clinical trials. Trials for the feasibility of using carefully validated multiplex genetic testing to determine therapeutic choice have been initiated for early-staged tumors, but getting the results of those trials will take time. In the coming decade, results of those randomized trials will be published and will affect our decisions.

With the broader availability of tumor tissues associated with clinical data, development of patient-specific tumor models, rapid understanding of therapeutic resistance mechanisms, and the development of pathway imaging, it will be possible to optimize the targeted therapy approach to most tumor-driver pathways. The potential list of approved targeted agents in cancer is likely to increase over the next 10 years.

In conclusion, it is my opinion that targeted agents will be used to treat most tumors within the next decade, but will not be ready for all.

References
Over the last decade, a better understanding of the molecular biology of cancer and the recognition of the existence of “driver mutations” upon which many cancers depend for survival led to the clinical development of novel therapeutic agents that specifically target pivotal signaling pathways dysregulated in tumor cells. In contrast to traditional chemotherapy, which kills all rapidly dividing cells without selectivity, molecularly targeted agents (MTAs) act against specific molecules involved in cancer cell growth and survival. The approval of trastuzumab, for the treatment of HER2-overexpressing breast cancer,\(^1\) and imatinib, for the treatment of chronic myelocytic leukemia featuring a BCR-ABL translocation and gastrointestinal stromal tumors with selective c-KIT oncogene-activating mutations, at the turn of the last century heralded the start of this new era of molecular oncology.\(^2,3\)

Although chemotherapies remain, for now, the backbone of current cancer treatment, the development of MTAs represents a major breakthrough. Currently, there are a number of different MTAs approved by the US Food and Drug Administration to treat different types of cancer. Many more are being tested and a number of new identified targets are forthcoming. We are moving from the traditional “one size fits all medicine” to the new paradigm of “personalized medicine” that delivers the right care to the right patient at the right time.

Personalized oncology includes the concept that each individual solid or hematologic malignancy in each person is unique in cause, rate of progression, and responsiveness to treatments. We have to stop searching for a single drug to treat all patients with a specific indication and start looking at the different patients, who need to be treated differently.

Thanks to recent advances in biology and in high-throughput genomic profiling technologies, hundreds of genomic alterations have already been recorded, and our knowledge of these changes is growing. Malignant diseases are no longer classified only by site and histology, but are separated into various homogeneous molecular subtypes, distinguished by a presumed key molecular alteration. A paradigmatic example is represented by the history of lung cancer. Standard cisplatin-based chemotherapy has been traditionally associated with relatively low response rates, significant toxicities, and poor survival. After decades of small, albeit significant, improvements, the discovery of oncopgenic drivers and the development of drugs able to specifically target these alterations have produced a substantial revolution in the management of patients carrying these “druggable” mutations. Epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) have become a mainstay in treatment of non–small cell lung cancers (NSCLCs) harboring EGFR-activating mutations, with the median survival for this subgroup of patients now greater than 2 years\(^4,5\) and more than twice that of patients receiving only chemotherapy.\(^6\) More recently, another TKI, crizotinib, has shown potent antitumor activity in patients with lung tumors bearing rearrangements of the anaplastic lymphoma receptor tyrosine kinase (ALK) gene, leading to a spectacular 57% response rate and a 90% disease control rate.\(^7\)

However, the use of MTAs raises some major challenges. One is represented by the relatively small proportion of patients that can benefit from those treatments. For example, EGFR-activating mutations are present in less than 10% of all NSCLC patients, ALK rearrangements in only 4%-5%.

In the next decade, oncology will move definitively from a reactive to a proactive discipline that is predictive, personalized, preventive, and participatory with the ultimate goal to understand entirely the complexity of biological networks that govern carcinogenic processes and to harness this information to provide better patient care. The toughest challenge that we have now involves the reorganization of health care systems in order to absorb the cost of novel methods and targeted treatments, ensuring individuals receive the best care.

References
Over the past decade or so, we have made remarkable progress in defining the molecular basis of cancer and leveraging that knowledge in the selection of appropriate, rational, and targeted therapies for individual patients. At the basic science level, we have built a sophisticated picture of the underlying molecular, cellular, and immunological hallmarks of cancer. One of the most exciting advances is the recognition of the fundamental role that cellular metabolism plays in fueling tumorigenesis. At the genomic level, we have seized upon the historic foundation of the Human Genome Project to construct a high-resolution atlas of the genomic landscape of cancer-related mutations, not only to further basic research, but also to provide personalized diagnostics to individual patients. Increasingly, we are detailing and describing cancer at the molecular level, not merely by the site of origin. As we do so, we can begin to grasp the complexity of the problem we are facing and, more immediately, expand the repertoire of molecular agents that we can deliver to patients. Our translational and clinical colleagues report many encouraging signs of success as new molecular entities strike powerful blows against cancer targets. And when resistance does emerge, we have other weapons that we can apply in combination. Perhaps the most exciting field at the moment is the area of immuno-oncology, a potential paradigm shift in the targeted therapy of cancer. As a firm contender in dismantling cancer’s armory, immunotherapy is already being applied and extended to more tumor types, and powerful immunotherapeutic agents are being combined with the current cornerstones of cancer therapy—chemotherapy and radiation.

Despite exciting progress at the lab bench and in the clinic, we as a community must be careful about giving false hope to patients and their families. As I reflect on the present scenario, my answer to the question at hand will have to be no, not all cancer patients will be treated by targeted therapies in the next 10 years. Our endeavors, based on the current promising weaponry against cancer must be carefully selected and focused as a community to ensure that we continue to expand our efforts and our successes to an increasing amount of patients. While we are making incredible progress, we must set our sights even higher by acting now in collaboration, at the international level. We must address, for example, the major loss of opportunity through important data not being suitably harnessed, shared, or stored.

Big data is everywhere, it’s part of everyday life and yet remains a stalled breakthrough in oncology. Huge amounts of data across patient populations and settings remain untapped, and consequently, embedding research into practice is not happening nearly as quickly as it possibly could. If we do not quite literally share to care, we cannot possibly hope to accelerate our collective efforts aimed at treating all cancer patients by targeted therapies. Determined action should focus on empowering researchers and clinicians to exploit this trove of biological knowledge and clinical data. If not, too many patients will still depend on the crude tools and approaches of the 20th century to arrest their cancer. We must continue to encourage early detection and prevention.

Concerning the former, detecting circulating tumor DNA or proteins for early-stage detection of cancer is undoubtedly poised to be another powerful tool. The potential and widespread application of this approach is, however, largely out of reach. Again, we must join forces to deliver on the promise of precision oncology. This, and other must-have conversations, including the much-debated topic of improved clinical trial design to balance speed and safety with real-time assessment of data and making fluid adjustments in dosage and treatment as needed, will largely depend on the collective, global consensus among our medical colleagues, payers and funding agencies, legislative bodies, and other key stakeholders.

In short, to sustain the considerable progress we have made, we must continue to invest in our hospitals, our medical centers, and most important of all, in the basic research that provides the foundation for our hopes of delivering truly targeted medicine.
Decades of basic research aimed at understanding the processes of carcinogenesis, cancer progression, and metastasis have fueled the development of novel therapeutic approaches in oncology. A new generation of anticancer drugs, including targeted therapies and immunotherapies, are reaching a stage where they are becoming available for cancer patients. Servier is determined to play a significant role in this therapeutic revolution. The current review summarizes ongoing studies and future areas of development of Servier’s armamentum to fight cancer.

Cancer is a complex multifactorial disease. In order to understand the diversity of neoplastic diseases, Hanahan and Weinberg have proposed a logical framework of ten biological capabilities—referred to as hallmarks—acquired by tumor cells during their multistep transformation. These include sustained proliferative signaling, induction of angiogenesis, resistance to apoptosis (programmed cell death), and hijacking of the immune system for survival and metastasis. This improved knowledge is allowing us to fight cancer by targeting the molecular pathways responsible for these acquired capabilities. Unfortunately, however, when targeted therapies are directed toward a single one of these hallmarks of cancer, a few cancer cells will often manage to survive and eventually the tumor adapts and escapes treatment. Indeed, this explains why, even though targeted therapies often report dramatic rates of tumor response, the majority of cancers eventually progress and become refractory to treatment. Servier is therefore convinced that durable clinical benefit can only be achieved by using either drugs targeting multiple pathways or combined therapies.
Starting from these considerations, Servier’s International Research Institute (IRIS) has built a diversified pipeline of innovative products targeting multiple and complementary cancer hallmarks. Servier’s research and development activity is organized around three axes: molecules targeting receptors with tyrosine kinase (TK) activity, molecules able to restore apoptosis in cancer cells, and molecules harnessing the immune system to fight cancer. In addition, Servier currently commercializes two cytotoxic products: Muphoran® (fotemustine) and Pixuvri® (pixantrone). To optimize drug combination and accelerate clinical development, Servier has leveraged an extensive network of biotechnology and industrial partners with recognized track records in oncology. To maximize the efficiency of its discovery programs, Servier has also developed scientific collaborations with many of the most prestigious research institutes in oncology.

Pixantrone

Pixantrone (Pixuvri®) is a novel aza-anthracenedione, and was initially developed by CTI BioPharma. This new treatment partially inhibits topoisomerase II and forms stable, covalently bound DNA adducts, thereby preventing DNA replication and transcription that ultimately induces mitotic infidelity and catastrophe. Unlike classical anthracyclines, pixantrone has little cardiotoxicity: no contraindication is stipulated within the summary of product characteristics; there is no exclusion for patients with previous anthracyclines, no cumulative dose restrictions apply. This means that it can be administered to patients who have had near maximal lifetime exposure to anthracyclines. CTI BioPharma and Servier have recently signed an exclusive license and collaboration agreement for the commercialization and further development of pixantrone.

In 2012, pixantrone obtained a conditional marketing authorization in the European Union as a monotherapy to treat patients with multiply relapsed or refractory aggressive non-Hodgkin B-cell lymphoma (NHL), such as diffuse large B-cell lymphoma (DLBCL). This authorization was granted following a phase 3, multicenter, open-label, randomized trial in 140 patients with aggressive NHL. In this trial, patients were randomly allocated to up to six cycles of pixantrone (50 mg/m² base intravenously on days 1, 8, and 15 of a 28-day cycle) or physician’s choice of treatment (administered according to the manufacturer’s instructions on dosage and schedule). Significantly more patients in the pixantrone group achieved complete or unconfirmed complete response by the end of the treatment period: 14 patients (20%) vs 4 patients (6%) of the comparator group (P=0.021). The median duration of these responses was 9.6 months in patients given pixantrone compared with 4 months in the comparator group. There was also a significantly better rate of overall response with pixantrone (37% vs 14%; P=0.003), which translated into a prolongation of progression-free survival (5 months vs 2.6 months; P=0.0035). These highly promising results in relapsed or refractory patients were obtained against an acceptable safety profile, supporting the use of pixantrone as a single-agent therapy for the care of these difficult patients.

CTI BioPharma and Servier are currently conducting a confirmatory phase 3 trial in patients with aggressive NHL to compare pixantrone versus gemcitabine when both drugs are used in combination with rituximab. Further studies will determine whether pixantrone could also be applied as a second-line therapy or in combination with various novel and targeted therapies. Pixantrone is currently commercialized in various European countries, including Germany, France, and the United Kingdom.

Tyrosine kinase inhibitors

Lucitanib

Lucitanib (S80881) is an orally bioavailable TK inhibitor currently being codeveloped worldwide by Servier and a US-based company Clovis Oncology, and in China with the Shanghai Institute of Materia Medica (SIMM). Lucitanib is a potent and selective inhibitor of the fibroblast growth factor receptors (FGFR) 1, 2, and 3, the vascular endothelial growth factor receptors (VEGFR) 1, 2, and 3, and the platelet-derived growth factor receptor (PDGFR) α and β. At the present time, it is being developed in hormone-sensitive breast cancer and advanced squamous cell lung cancer patients. The activity of lucitanib in other solid tumors is currently evaluated in phase 1 studies. Preclinical and clinical studies have established that lucitanib inhibits tumor angiogenesis. Angiogenesis is the process by which new blood vessels are produced, and is essential for growth once a tumor reaches a certain diameter. Indeed, in the absence of new blood vessels, tumor cells die from nutrient starvation, metabolite poisoning, and hypoxia, thereby constituting a potential target for anticancer therapy. VEGF is the main driver of angiogenesis in human tumors. However, it has been shown in clinical studies that when cancer patients were treated with bevacizumab (an anti-VEGF antibody), other proangiogenic factors, such as FGF and PDGF
remained active or even became upregulated; high serum levels of FGF or PDGF in bevacizumab-treated patients were associated with a poorer prognosis. In this context, it has been suggested that the FGF/FGFR and PDGF/PDGFR pathways underly the mechanisms of resistance to VEGF inhibitors. As lucitanib inhibits VEGFR, FGFR, and PDGFR, it is a particularly promising candidate to counteract resistance to therapies targeted uniquely to VEGF/VEGFR. Tumor-infiltrating myeloid cells also regulate angiogenesis. Inhibition of the colony-stimulating factor 1 receptor (CFS1R), another target of lucitanib that is also known as macrophage colony-stimulating factor receptor [M-CFSR]), prevents hypoxia-induced infiltration of macrophages and may reinforce the antiangiogenic properties of lucitanib.

In addition to its effect on tumor vasculature, lucitanib may also act directly on cancer cells via the inhibition of FGFR. Indeed, FGFR is frequently amplified in human tumors, especially from breast or lung. In these tumors, FGF secretion by either the cancer cells or the surrounding stroma contributes to tumor growth through an autocrine or paracrine loop. The direct activity of lucitanib on FGFR expressed by cancer cells has been observed in in vitro experiments and in animal models.

Lucitanib has been tested for the first time in humans in a phase 1/2a study, which demonstrated clinical benefit in both FGF-aberrant and angiogenesis-sensitive populations. Between June 2010 and September 2012, this open-label study included 76 patients with solid tumors (17 in the dose-escalation and 59 in the expansion phase). Nineteen patients had breast cancer (25%), 11 had colon cancer (14%), 9 had thyroid cancer (12%), and 7 had lung cancer (9%); 42% had greater than three lines of previous chemotherapy. Lucitanib has shown clinical activity in a variety of tumor types, with an objective RECIST (Response Evaluation Criteria In Solid Tumors) response rate of 28%, a disease control rate of 80%, and several durable responses and long-lasting stabilizations, with all patients having previously received at least three lines of chemotherapy. Notably, half of patients with FGF-aberrant breast cancer (ie, 6 patients out of 12) achieved partial response during the study with a median progression-free survival close to 10 months. Toxicity was mainly related to its antiangiogenic properties, with hypertension and proteinuria, which are well-known side effects of angiogenesis inhibition. Whether direct inhibition of FGFR expressed on cancer cells contributes to tumor regression in breast cancer patients is currently being investigated in a phase 2 trial (FINESSE).

◆ S49076
S49076 is another TK inhibitor currently in development for the treatment of glioblastoma and non–small cell lung cancer. S49076 is an orally bioavailable inhibitor of the hepatocyte growth factor receptor cMET, the growth arrest-specific 6 (GAS6) receptor AXL, as well as FGFR 1 and 2. It appears to be particularly suitable for the management of patients who are resistant to specific targeted therapies or chemotherapies. The enthusiasm for targeted therapies has been tempered by the observation of frequent and rapid relapses following good initial response in the designated subpopulation of patients. For example, lung cancer patients treated with inhibitors of epidermal growth factor receptor (EGFR), such as gefitinib or erlotinib, experience clinical response that rarely lasts more than 2 years. These relapses are often due to the selection of cancer cells that overexpress other TK, thereby short-circuiting the effects of EGFR inhibition. In this context, we note that the most commonly upregulated TK receptors include cMET, AXL, and FGFR. Insofar as S49076 targets exactly these three TK receptors, we expect this agent to have the potential to address this important medical need. Similar mechanisms have been described for the onset of resistance to VEGFR inhibition or various chemotherapies.

More generally, the activation of cMET, AXL, and FGFR pathways is associated with epithelial to mesenchymal transition, cancer cell dissemination, and metastasis. We should recall that some chemotherapy drugs appear to induce objective responses, but fail to confer any survival benefit. This has been shown to be due to the selection of cell variants that have undergone epithelial to mesenchymal transition and display a more aggressive phenotype. Combination of chemotherapy with S49076 may have a substantial preventive effect on this phenomenon.

The first study of S49076 in humans demonstrated that it has a remarkable safety profile, which is essential for a product whose principal benefit is expected to be found in combination with other anticancer agents. In summary, S49076 will allow us to target a number of critical steps for cancer progression, and is an agent with great promise.

◆ S81694
S81694 is a member of the pyrazoloquinazoline family and is a specific inhibitor of the cell cycle checkpoint kinase TTK, also known as MPS1 (monopolar spindle 1). TTK plays a critical role in the control of mitosis regulating the spindle assembly checkpoint (SAC) through proper kinetocore recruitment of other essential SAC proteins. The SAC complex regulates a mitotic mechanism required for proper chromosome alignment, influencing the stability of the kinetochore–microtubule interaction and ensuring that cells do not divide until all sister chromatids correctly align to the metaphase plate. Failure to assemble the SAC complex results in unbalanced chromosome separation, leading to aneuploidy and cell death. TTK is highly expressed in fast-dividing cells, including virtually all cancer cells. Triple-negative breast cancer and acute myelocytic leukemia are among the tumor types that are the most dependent on the activity of TTK. Unlike mitotic inhibitors, such as taxanes and vinca alkaloids, TTK inhibitors accelerate cell division and therefore represent a novel mechanism to target fast-dividing cells.
The new agent, S81694, is a specific ATP-competitive inhibitor of TTK. It was initially discovered by Nerviano Medical Science (as a follow-up of NMS-P715) from whom Servier acquired the patent in 2014. S81694 will enter clinical development in solid tumors the second quarter of 2015.

**Inducers of apoptosis in cancer cells**

◆ **S55746**

Evasion from apoptosis (programmed cell death) is one of the hallmarks of cancer, ie, one of the most frequent alterations of cancer cells responsible for carcinogenesis. Apoptosis is also one of the most frequent mechanisms of escape from chemotherapy or targeted therapies. Proteins of the B-cell lymphoma 2 (Bcl-2) family are crucial inhibitors of apoptosis, and are therefore regarded as prosurvival factors. They act by sequestering and antagonizing the inducers of apoptosis BAX and BAK. Inhibiting the prosurvival members of the Bcl-2 family is expected to restore the competence of cancer cells for apoptosis. On the other hand, apoptosis is also a physiological process and, until recently, the clinical application of such inhibitors was hindered by the difficulties in designing molecules that could prevent specific interactions between BAX, BAK, and antiapoptosis proteins. The strong homology within the Bcl-2 family of proteins initially led only to the development of drugs that blocked several members of the protein family, which gave rise to undesirable side effects, mostly platelet toxicity due to Bcl-xL inhibition, and limited clinical application.

A new collaboration between Servier and Vernalis was set up in 2007 with the aim of discovering new specific inhibitors of the main members of the Bcl-2 family of proteins. In 2014, the first of these molecules, S55746, a Bcl-2–specific inhibitor, entered clinical development. A month later, Servier and Novartis signed a global collaboration agreement for the development and commercialization of S55746.

◆ **Other inducers of apoptosis**

Further inhibitors of the prosurvival members of the Bcl-2 family of proteins are currently in preclinical development at Servier. Notably, Servier has discovered an inhibitor of Mcl-1, one of the most important members of this family that is not yet targeted. Consistent preclinical data show that Mcl-1 inhibitors are highly active against models of hematological and solid tumors, either as single agents or in combination with approved targeted therapies.

**Immunotherapy**

Cancer cells develop complex interactions with mesenchymal cells, as has been observed in in vivo models in both patients and animals. In solid tumors (including lymphomas), these mesenchymal cells are organized and are collectively referred to as the tumor stroma. The tumor stroma, which contains activated fibroblasts, endothelial cells, and immune leukocytes, is essential for tumor growth, cancer dissemination, colonization of distant organs (ie, metastasis), and resistance to treatment. Importantly, solid tumors contain many types of immune cells, including macrophages, neutrophils, lymphocytes, and various myeloid-derived suppressor cells, whose number, localization, polarization, and activation status are some of the best predictors of patient survival. In recent years, targeting the interactions between cancer cells and tumor-infiltrating leukocytes (TILs) was recognized as a promising strategy to fight cancer. In the recent years, the US Food and Drug Administration (FDA) approved two novel drugs, sipuleucel-T (Provenge® in 2010) and ipilimumab (Yervoy® in 2011), which directly harness the immune system against cancer, and are being applied in advanced prostate cancer and un-resectable or metastatic melanoma, respectively.

More recently, adoptive therapies using genetically modified autologous T lymphocytes have shown impressive results in acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL). Immunotherapy via agents such as these is one of Servier’s priorities in oncology.

**Engineered antibodies**

In collaboration with the US-based biotech MacroGenics, Servier is developing a number of engineered antibodies, including a humanized, B7-H3–specific IgG1 (immunoglobulin G 1) with an Fc domain optimized for enhanced immune effector functions like antibody-dependent cell cytotoxicity and several Dual-Affinity Re-Targeting (DART) antibodies. All these antibodies are first in class. The most advanced DART antibody recognizes both CD3 expressed on T lymphocytes and CD123 expressed on acute myelocytic leukemia and myelodysplastic syndrome cells. When this dual binding occurs on T lymphocytes and cancer cells, it stabilizes the formation of conjugates between cancer cells and lymphocytes, and leads to T-cell receptor aggregation and lymphocyte activation. This results in tumor cell lysis.

The power of this approach is that any lymphocyte, irrespective of its original antigen specificity, can be mobilized against tumor cells, leading to a very strong immune reaction. Servier also has an option on a number of other DART antibodies discovered by MacroGenics, including a gpA33 x CD3 bispecific one for the treatment of colorectal cancers.

**Cell therapy**

Servier is currently collaborating with the French biotech Cellectis to develop and commercialize a UCART19 product candidate. The use of autologous T cells engineered to express chimeric antigen receptors (CARs, a genetic fusion between a tumor antigen binder, an intracellular T-cell activation domain, and a costimulation signal) has recently emerged as a powerful approach to treat patients with advanced CLL and ALL. The production of autologous CAR-expressing T cells requires a long and tedious process that starts with the purification of a patient’s own T cells.
Following this, the cells are genetically modified to express the tumor-specific CAR. The transduced cells are then purified and amplified, and finally transferred back to the patient. This process has to be repeated for each patient, therefore making this a true personalized medicine.

The goal of the UCART19 (universal CAR T) program, developed by Cellectis and for which Servier has an exclusive option to license, is to modify this process so that T cells from healthy donors, instead of those derived from the patient, can be used as the starting material for this treatment. Consequently, a single preparation of modified lymphocytes (universal CAR T cells or UCART cells) could be used for several, and possibly hundreds of, patients. The hope is that UCART cells could become an “off-the-shelf” drug, with great promise for patients. The novelty here is the use of Cellectis proprietary gene editing technology TALEN®23 to inactivate the endogenous T-cell receptor gene expressed by the starting lymphocytes, thereby preventing the induction of graft-vs-host disease. This will also require proper conditioning of the patients to allow for sufficient UCART lymphocyte persistence in an allogeneic patient. The development of the UCART technology represents significant manufacturing and clinical challenges, but it would also be a game-changer making this promising approach available for large cohorts of patients.

Collectes is currently developing UCART19 cells targeting CD19, an antigen expressed by CLL and ALL cells, which was successfully targeted in the previously conducted autologous CAR trials. Collectes has also started to construct new CARs targeting different receptors expressed in various types of cancer, including solid tumors.

**Conclusion**

Servier’s portfolio in oncology is growing rapidly. The most advanced products comprise novel cytotoxics and thymidine kinase inhibitors targeting some of the most dramatic cancers: glioblastoma, non-Hodgkin lymphomas, melanoma, and breast and lung cancers. In the future, Servier will further develop its efforts on inducers of apoptosis and immunotherapies, which we believe carry potential in a large set of indications and could efficiently complement current standards of care. This activity is leveraged by a network of partnerships that includes some of the big names of global oncology and also a number of smaller partners from the field of biotechnology. Servier’s ambition is to bring new products and new therapeutic options to the many who suffer from cancer.

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**References**


**Keywords:** apoptosis; breast cancer; cell therapy; glioblastoma; immunotherapy; leukemia; non-Hodgkin lymphoma; targeted therapy
PRODUITS EN DÉVELOPPEMENT EN ONCOLOGIE CHEZ SERVIER

Le développement de nouvelles approches thérapeutiques en oncologie s’est nourri pendant des dizaines d’années de la recherche fondamentale visant à comprendre les processus de la carcinogenèse, de la progression du cancer et des métastases. Une nouvelle génération de produits anticancéreux, dont les traitements ciblés et l’immunothérapie, devient maintenant disponible pour les patients cancéreux. Servier est décidé à jouer un rôle significatif dans cette révolution thérapeutique. Cet article résume les études en cours et les futurs domaines de développement de son portefeuille pour lutter contre le cancer.
Over the last decade, early phase trials in oncology have switched from the “one-size fits all” approach of the cytotoxic chemotherapy area, to a “personalized and precision medicine” strategy based on molecular selection for patients receiving novel molecularly targeted agents (MTAs). This patient-centered approach has revolutionized the role of phase 1 trials, which are now not only dose-finding studies, but also hypothesis-testing trials aiming at making the proof of concept of a given biological rationale. As such, many paradigms of the traditional drug development model have been challenged by novel stakes including molecular enrichment, early development of companion biomarkers, consideration of late or cumulative toxicities, appropriate recommended phase 2 dose definition and customized phase 1 trial designs for MTAs. Consequently, the role of early phase trials in accelerating the drug development and making it more efficient is increasingly important, and the smart design of phase 1 trials can now strongly influence the “go/no-go” decision for further drug development. In this issue of Medicographia, I will discuss the current stakes of early clinical drug development in oncology, and give tracks for improving and fostering the success of this process.
What are the clinical stakes of the early clinical drug development in oncology?

Currently, the most important clinical stakes of the early clinical drug development in oncology are to smartly adapt the phase 1 trials to novel MTAs and to favor hypothesis-testing trials, ie, based on a specific preclinical rationale, while keeping the costs and duration of early phase studies financially and ethically suitable.

Indeed, the advent of MTAs over the last 15 years has revolutionized the early phase trials landscape. If the primary end point of phase 1 trials should remain the determination of the recommended phase 2 dose (RP2D) and description of the drug safety, early phase trials now also play a pivotal role in multiple other fields that will be key for the success of later drug development. Notably, the “one-size fits all” approach, which does not take into account the patient-to-patient molecular variability of the tumor, is not acceptable anymore in the context of MTAs. Moreover, as illustrated by the strikingly high attrition rate of the number of drugs developed between phase 1 and phase 2 trials, and by the proportion of drugs that still fail late in their development, there is an urge to improve early phase trial efficiency.

MTAs, which now represent the vast majority of drugs that come into early phase trials, have challenged many paradigms that were established in the era of conventional cytotoxic chemotherapy (CCC) and brought new challenges, such as the need for selecting the right patients for molecular enrichment, the necessity to have access to the tumor for molecular analysis, the difficulty in determining the optimal dose and schedule, the importance of early development of companion biomarkers, the pitfall of the feasibility of drug combinations, and the dilemma of the choice of the most appropriate method and criteria for assessing tumor response.

Overall, I think that phase 1 trials face new stakes that have now been well identified, but have not yet been appropriately addressed in the current clinical practice. This relative delay in modifying phase 1 trials designs and the way such studies are carried out, is currently impacting on the success of some drugs’ development. Therefore, it is now crucial to optimally design all phase 1 trials as true “hypothesis-driven” and “hypothesis-testing” trials, by considering the above-mentioned points, prior to starting the clinical evaluation.

What makes early clinical drug development in oncology efficient?

First of all, and prior to any first-in-human phase 1 trial, very robust preclinical data must be obtained in relevant and appropriate preclinical models. At the clinical stage, the thorough consideration of all the challenges and stakes previously mentioned will make early clinical drug development efficient. The right patient selection, the early development of companion biomarkers (including predictive biomarkers, pharmacokinetic [PK] and pharmacodynamic [PD] biomarkers as well as other intermediate or molecular biomarkers), the search for noninvasive access to tumor material and the appropriate choice of the response evaluation method and criteria are all key elements of successful early drug development. Furthermore, other important aspects should be considered, such as the choice of an appropriate design, which will favor both a rapid determination of the maximum tolerated dose (MTD) and RP2D (to minimize the number of patients included at low and potentially inefficient doses), and favor the inclusion of a sufficient number of patients at doses known to be safe and at which the target is modulated, so that more information is obtained about interpatient variability. Moreover, molecular enrichment (ie, the inclusion of patients whose tumor presents the molecular aberration of interest) should be favored, as this proportion currently remains insufficient.3 Phase 1 tri-

**Selected abbreviations and acronyms**

- ALK: anaplastic lymphoma kinase
- CCC: conventional cytotoxic chemotherapy
- MTA: molecularly targeted agent
- MTD: maximum tolerated dose
- PD: pharmacodynamic
- PK: pharmacokinetic
- RP2D: recommended phase 2 dose.
als should also incorporate clear and early "go/no-go" decision rules, so that the evaluation of inefficient or too toxic compounds is terminated early. For example, molecules that have not delivered any signs of activity at the end of the phase 1 trial, despite an appropriate patient selection, should not be further investigated, even in combination.

Finally, mechanisms of resistance should be studied as early as at the phase 1 trials, so that preclinical research about how to overcome and bypass them can be started while later phase trials are being performed.

**How do targeted therapies change the situation?**

Targeted therapies have dramatically changed the situation for several aspects. First, patient selection has become crucial. Indeed, most phase 1 trials are now based on robust preclinical data, and as such, should be designed as "hypothesis-driven" and "proof-of-concept" trials rather than only dose-finding studies. This is well illustrated by the recent study published by Tsimeridou et al, which reported that the likelihood of experiencing some degree of response was almost four times higher in patients receiving a drug matched to their tumor's molecular aberration, than in patients receiving an unmatched drug (29% vs 8%, respectively). This implies the parallel development of several companion biomarkers, and notably predictive biomarkers, which will allow selection of the right patient population. Phase 1 trials play a key role in the development of such biomarkers, as they will bring the first clinical data on assays that will require further validation in later phase trials prior to approval for clinical use. Beyond the development of an appropriate selection biomarker, tumor accessibility is a key stake of hypothesis-driven trials in the context of personalized medicine.

If archived tumor material is usually available for almost all patients, it does not represent the optimal material to use for patient selection: indeed, the molecular profile of the tumor can vary over time and frozen material, which is required for some molecular diagnostics, is usually not available. A first option is then to biopsy the patient's tumor prior to starting the phase 1 trial (and ideally also when resistance occurs), but this is not always feasible or simply accepted by the patient. Therefore, liquid biopsies, including the analysis of circulating tumor DNA and circulating tumoral cells, represent a very promising option for overcoming this recurrent issue. Besides the patient selection challenge, the determination of the dose that should be used in phase 2 trials is a real stake of current early phase trials evaluating MTAs. Indeed, the MTD is not always reached with such agents, and the determination of the RP2D is consequently sometimes based on alternative parameters, such as the maximum feasible dose, or PD and PK data (optimal biological dose). This highlights the importance of having real-time access to PK and PD data as these can be key, not only for the dose-escalation process, but also for the dose-recommendation process. Also, the method of determination of the RP2D should evolve to better correspond to the MTAs' schedule of administration and pattern of toxicities. Indeed, as these agents are due to be administered for a prolonged period of time, late toxicities (ie, occurring after the dose-limiting toxicity period), cumulative toxicities, and prolonged moderate toxicities should deserve more attention. As a matter of fact, such toxicities seriously threaten the quality of life of the patient and can impact on patient's observance at later stages of drug development. This challenge is well-illustrated by the recent publication of Fontes-Jardim et al, which notably reports the poorer ability of phase 1 trials of MTAs to predict the dose that will be used in phase 3 trials or the dose at which the drug will be approved, as compared with phase 1 trials evaluating CCC. To further investigate this issue, guidelines and recommendations have very recently been elaborated by a European Organisation for Research and Treatment of Cancer (EORTC)–led task force of international phase 1 experts, and which are now publicly available. One major point of these recommendations is that the RP2D should be determined based on achieving >75% of the intended relative dose-intensity, rather than being based on a certain proportion of severe toxicities.

Another challenge that phase 1 trials are now facing is the choice of the optimal technique and criteria for tumor response assessment. Indeed, if Response Evaluation Criteria In Solid Tumors (RECIST) criteria and computed tomography scans have proven useful and efficient for most—if not all— CCC, MTAs sometimes cause true responses, which are either delayed or not evaluable by RECIST criteria. Examples include pseudoprogressions or prolonged stable diseases observed with gastrointestinal stromal tumors (GISTs) treated with c-kit inhibitors, which are better evaluated by Choi criteria, changes in tumor perfusion and vasculature under antiangiogenic agents, which are better evaluated by contrast ultrasound or perfusion magnetic resonance imaging, or more recently, pseudoprogressions or delayed responses observed under immunotherapies, which have led to the development of specific criteria, namely the immune-related Response Criteria (irRC). Therefore, the response evaluation method and criteria should be thoroughly chosen in phase 1 trials, as the detection of activity of the drug will be key for the "go/no-go" decision of the drug development in later phase trials.

Other stakes of early clinical drug development include the smart and relevant development of drug combinations: indeed, combining an MTA with a CCC or with another MTA has proven more challenging than expected, both in terms of toxicity management and determination of the optimal administration schedule or dosage. Another upcoming challenge will be a smart combination with immune checkpoint inhibitors. Moreover, efficacy results of combination trials have sometimes been disappointing, and a lot remains to be learned in this field.
In a nutshell, MTAs do considerably change the landscape of phase 1 trials and most of the required amendments to the current uses and practice have now been well identified. This has been followed by the elaboration of guidelines or recommendations that are unfortunately still inconstantly followed, but have encouragingly been increasingly implemented in recent phase 1 trials.

**Does phase 1 trial design have to be amended?**

Yes, phase 1 trial design should be amended in order to better address the above-mentioned points and better correspond to the MTA landscape. Although important evolutions are required, the skeleton of phase 1 trials should remain unchanged: the primary end point should still be the evaluation of the drug safety profile and RP2D; phase 1 trials should also still encompass one dose-escalation and one dose–expansion phases (although the design of each of them could be modified); and finally, the dose-expansion phase should still focus on fine-tuning the recommended dose, rather than on evaluating efficacy.

However, major changes could be made to improve current phase 1 trials. First, model-based designs are still too rarely used, mainly because the “3+3” design is easy to work with, and has been doing well over years. The “accelerated titration,” “modified toxicity probability interval (MTPI),” or “Bayesian” designs may be more appropriate. Also, some provisions should be made to facilitate both the enrolment of patients whose tumor harbors the molecular alteration of interest, as well as the enrolment of patients at doses known to be safe and that display both a PD effect and the desired PK profile.

In order to best allow this enrichment in molecularly selected patients, a smart collaboration between participating centers should be favored, rather than systematic competitive enrollment of molecularly unselected patients.

Second, the traditional phase 1 eligibility criteria should be revisited and customized for each phase 1 trial rather than reproduced from one trial to another. For example, systematically excluding patients having already received compounds targeting the same pathway, patients with brain metastasis, or patients with moderate renal or liver dysfunction, is not relevant, and eligibility criteria should be thoroughly defined to best match the agent evaluated.

Third, recommendations that have recently been established to best define the RP2D in phase 1 trials of MTAs, should be followed whenever possible. This includes notably revisiting all toxicities (whatever their cycle of occurrence) described during the trial prior to determining the next dose, when completing a given dose level, as well as targeting the delivery of >75% of the intended dose intensity when defining the RP2D.

Altogether, such design modifications should foster early drug development in oncology and make this process more efficient for MTAs, not only at the phase 1 step, but also for later steps of the compound development.

Finally, it is very important to realize that modern phase 1, in which the tested compound demonstrates clear signs of clinical activity, can be expanded up to hundreds of patients. In that regard, phase 1 trials testing new anaplastic lymphoma kinase (ALK) inhibitors (ie, crizotinib and ceritinib) have both enrolled over 250 patients. Phase 1 trials testing programmed cell death (PD-1) or programmed death ligand 1 (PD-L1) antibodies have enrolled a minimum of 200 patients (nivolumab) and as many as 1100 patients (pembrolizumab). These changes are illustrated in Figure 2.

**How may phase 1 accelerate regulatory approvals of new expected anticancer drugs?**

Recent successful phase 1 trials illustrate very well how early phase trials can accelerate regulatory approvals of new anticancer drugs. Such examples include phase 1 studies evaluating olaparib (AZD2281, Astra Zeneca; a poly ADP-ribose polymerase [PARP] 1/2 inhibitor), vemurafenib (PLX4032, Plexxikon; a B-raf inhibitor), crizotinib (PF-02341066, Pfizer; an ALK inhibitor), and the hedgehog inhibitor vismodegib (GDC-0449, Roche).
these studies, the role of predictive biomarkers for response has been pivotal in the hypothesis-testing study design and provisions have been made to favor the inclusion of molecularly selected patients whose tumors presented the molecular alteration of interest. Consequently, the proof of concept and activity of the drug detected in phase 1 trials has, in some cases, even been sufficient to justify the procurement of a conditional approval from the US Food and Drug Administration (FDA), based on the results of the phase 1 only. Crizotinib and Vismodegib are the best examples of success of this strategy, with accelerated timelines for approval, ie, approximately 5 years only between the beginning of the phase 1 trials and the regulatory approval.

We therefore believe that enrichment strategies, as early as phase 1 trials, are definitely key in accelerating drug development in oncology. To favor this, phase 1 centers need a very large panel of readily available phase 1 and phase 2 trials (or networks of partners to which the patients could be referred) to optimally deal with the diversity of available targets.

References

Keywords: molecularly targeted drug; oncology; targeted therapy

**ENJEUX CLINIQUES DU DÉVELOPPEMENT PRÉCOCE DES MÉDICAMENTS EN ONCOLOGIE**

Ces 10 dernières années, les études de phase 1 en oncologie sont passées d’une approche « unique » de la chimiothérapie cytotoxique à une stratégie « de médecine précise et personnalisée » basée sur une sélection moléculaire pour des patients recevant de nouvelles thérapies moléculaires ciblées (TMC). Cette approche centrée sur le patient a révolutionné le rôle des études de phase 1, qui sont maintenant non seulement des études de recherche de doses, mais aussi des études de vérification d’hypothèse dont le but est d’établir la faisabilité d’un fondement biologique donné. C’est ainsi que de nombreux exemples de modèles traditionnels de développement des médicaments ont été contestés par de nouveaux enjeux dont l’enrichissement moléculaire, le développement précoce des biomarqueurs compagnons, la prise en compte des toxicités tardives ou cumulatives, la détermination de la dose de phase 2 recommandée appropriée et les schémas personnalisés des études de phase 1 pour les TMC. Par conséquent, le rôle des études de phase 1 pour accélérer le développement des médicaments et pour les rendre plus efficaces est de plus en plus important et la conception intelligente de ces études peut maintenant influer fortement sur la décision de poursuivre ou non le développement d’un médicament. Dans cet article de Medicographia, je vais analyser les enjeux actuels du développement clinique précoce des médicaments en oncologie et je vais proposer des pistes pour améliorer et promouvoir le succès de ce processus.
Supportive care: bringing better cancer treatment

by M. Aapro, Switzerland

Guideline-directed treatment, including that for supportive care, is cost reducing and improves patient outcomes, but recent developments increasing therapeutic modalities have introduced new types of toxicities; thus, new treatment algorithms will be required. We have already seen a positive impact on patient survival owing to granulocyte colony-stimulating factors, bisphosphonates, and possibly denosumab, to name a few examples. Furthermore, early palliative care has been shown to impact mortality due to cancer in 3 randomized studies. Despite the European Society for Medical Oncology’s (ESMO) position taken in 2003, calling for the development of optimal supportive and palliative care, patients unfortunately continue to be seen late in the course of illness. Nowadays, as delivering affordable medical care is a major challenge even for countries with a relative wealth of resources, one would imagine this would be more acutely felt in countries that are more limited in that regard. To best allocate their resources, such countries could adapt existing recommendations—such as those covering physical symptom management, pain management, monitoring and documentation, psychosocial and spiritual aspects of care, health professional education, as well as patient, family, and caregiver education—to priorities suggested by a consensus panel, similar to that suggested by the Breast Health Global Initiative.

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There is no doubt that we have made progress in controlling many symptoms related to classic cancer treatments, and consensus or guideline publications provide information for the clinician to improve patient care, both in younger and older patients. This is of course true only as long as guidelines are applied (Figure 1). When properly used, guideline-directed treatment is also cost reducing.

Nevertheless, new challenges are emerging for supportive care, as discussed recently in a commentary in the journal of the Multinational Association for Supportive Care in Cancer (MASCC). As stated by the authors, these challenges are exacerbated by the expected increase in the number and type of targeted therapies that will enter the oncologist’s arsenal based on new research and approvals. New treatment algorithms will be required, and there is a need to explore the management of these toxicities using novel approaches, including pharmacogenomics and genetic risk prediction in order to personalize management strategies.
Supportive care: bringing better cancer treatment – Aapro

FOCUS

An unforeseen impact of early palliative care
Among busy clinicians, interest for early palliative care was sparked by a study in patients with NSCLC that was published in 2010. The authors randomly assigned patients with newly diagnosed metastatic NSCLC to receive either early palliative care integrated with standard oncologic care or standard oncologic care alone. The primary outcome was the change in the quality of life (QOL) at 12 weeks, but the authors observed an unexpected impact on survival. Among the 151 patients who underwent randomization, median survival was longer in patients receiving early palliative care (11.6 months vs 8.9 months; P=0.02). Patients assigned to early palliative care had a better QOL than did patients assigned to standard care (mean score of 98.0 vs 91.5; P=0.03; on the Functional Assessment of Cancer Therapy – Lung scale [FACT-L], in which scores range from 0 to 136, with higher scores indicating better QOL). In addition, fewer patients in the early palliative care group had depressive symptoms, compared with the standard care group (16% vs 38%; P=0.01). A paper published by the same group showed that depression predicted worse survival in patients in that study with newly diagnosed metastatic NSCLC. Although early palliative care was associated with greater improvement in depression at 12 weeks, the data did not support the hypothesis that treatment of depression mediated the observed survival benefit from early palliative care.

The American Society of Clinical Oncology (ASCO) then put together a group that published a provisional clinical opinion on the topic in 2012. This evaluation of the available evidence (7 randomized controlled trials) about the benefits of palliative care in patients with metastatic cancer who are also receiving standard oncologic care concluded that survival benefit from early involvement of palliative care had not yet been fully demonstrated. However, the review panel concluded that palliative care—either combined with standard oncologic care or as the main focus of care—leads to better outcomes in patients and caregivers, and should be considered early in the course of illness for any patient with metastatic cancer and/or high symptom burden.

Other evidence for early palliative care in advanced cancer
Since then, 2 other studies have shown the importance of early access to supportive or palliative care. The first was a study conducted at the Princess Margaret Cancer Centre (Toronto, ON, Canada), between Dec 1, 2006, and Feb 28, 2011. A total of 24 medical oncology clinics were cluster randomized (in a 1:1 ratio, using a computer-generated sequence, stratified by clinic size and tumor site [4 lung, 8 gastrointestinal, 4 genitourinary, 6 breast, 2 gynecological]), to consultation and an at least monthly follow-up by a palliative care team (intervention group) or to standard oncologic care (control group). Eligible patients had advanced cancer, Eastern Cooperative Oncology Group (ECOG) performance sta-

**Supportive care: is there a survival benefit?**
Those who believe only in survival data will ask if this type of research is justified, looking at the potential impact of supportive care on patient survival. The answer is that a positive impact on survival is very likely in some cases, as shown in analyses about the proper application of granulocyte colony-stimulating factors. Also, it seems that a survival benefit is observed in patients treated with bisphosphonates in several settings, from multiple myeloma to postmenopausal patients with early breast cancer, and in a hypothesis-generating evaluation of denosumab in non–small cell lung cancer (NSCLC), for example. Clearly, one will find it difficult to show a survival impact of antiemetic studies, even if most professionals accept that nausea and vomiting are unacceptable side effects of oncological treatments.

**SELECTED ABBREVIATIONS AND ACRONYMS**

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<th>Acronym</th>
<th>Description</th>
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<tr>
<td>ASCO</td>
<td>American Society of Clinical Oncology</td>
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<td>EAPC</td>
<td>European Association of Palliative Care</td>
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<td>ESMO</td>
<td>European Society for Medical Oncology</td>
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<tr>
<td>FACIT-Sp</td>
<td>Functional Assessment of Chronic Illness Therapy – Spiritual well-being</td>
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<td>FACT-L</td>
<td>Functional Assessment of Cancer Therapy – Lung</td>
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<td>MASCC</td>
<td>Multinational Association for Supportive Care in Cancer</td>
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<td>NSCLC</td>
<td>non–small cell lung cancer</td>
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<td>ECOG</td>
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<td>BHGI</td>
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In that study, 461 patients completed baseline measures (228 in the intervention group, 233 in the control group); 393 completed at least 1 follow-up assessment. At 3 months, there was a nonsignificant difference in change score for FACIT-Sp between intervention and control groups (3.56 points; 95% confidence interval, −0.27 to 7.40; \( P = 0.07 \)), but differences in all other scales favored the intervention group. The authors concluded that this trial shows promising findings that support early palliative care for patients with advanced cancer.

The second study, which has just been published, investigated the effect of early versus delayed palliative care on outcomes, including QOL, symptom impact, mood, 1-year survival, and resource use (days in hospital/intensive care unit, emergency room visits, chemotherapy in last 14 days, and death location). Between October 2010 and March 2013, 207 patients with advanced cancer at a National Cancer Institute (NCI)-designated cancer center, a Veterans Affairs (VA) medical center, and community outreach clinics were randomly assigned to receive an in-person palliative care consultation, structured palliative care telehealth nurse coaching sessions (once per week for 6 sessions), and monthly follow-up either early after enrollment or 3 months later. The overall patient-reported outcomes were not statistically significant after enrollment (QOL, \( P = 0.34 \); symptom impact, \( P = 0.09 \); mood, \( P = 0.33 \)) or before death (QOL, \( P = 0.73 \); symptom impact, \( P = 0.30 \); mood, \( P = 0.82 \)). However, the Kaplan-Meier 1-year survival rate was 63% in the early group and 48% in the delayed group (difference, 15%; \( P = 0.038 \)).

**Does it matter?**

Though survival benefit results in supportive care studies are very encouraging to read about, are they necessary? Is it really important to show that quantity of life is influenced by supportive and palliative care interventions? Does anyone dispute the importance of pain control, which perhaps does not influence the quantity of life, but could influence the quality of survival? Is there really room for the never-ending dispute among healthcare professionals about supportive or palliative care? Back in 2003, the European Society for Medical Oncology (ESMO) had already taken a position calling for the development of optimal supportive and palliative care. However, despite the support given for the development of centers of excellence in this field, a recent survey has shown that palliative care facilities are used late in the course of disease, which is unsatisfactory. Data was generated from members of MASCC, ESMO, and the European Association of Palliative Care (EAPC), who completed the surveys online. A total of 62 program leaders completed the survey. Most programs had been in existence for over 5 years and were led by oncology-trained physicians who had an additional specialty. Most programs had consultative services and outpatient clinics with fewer having inpatient beds and institutionally associated hospices. Most programs provided patient continuity. Patients were generally seen late in the course of illness with the average survival of 23 days when seen as inpatients and 40 days when seen as outpatients.

**Now what?**

Clearly, the guidelines and consensus recommendations cannot be universal. Even countries with excellent resources are facing a crisis related to the costs of medicine. If these countries have limitations, what can be said about those with fewer resources? An example of one review addressing this issue is the Breast Health Global Initiative (BHGI), which has convened 3 expert panels to develop resource allocation recommendations for supportive and palliative care programs in low-income and middle-income countries. Each panel focused on a specific phase of breast cancer care: during treatment, after treatment with curative intent (survivorship), and after diagnosis with metastatic disease. The panel consensus statements—which cover physical symptom management, pain management, monitoring and documentation, psychosocial and spiritual aspects of care, health professional education, and patient, family, and caregiver education—were published in October 2013. Such panels may offer guidelines on how best to implement care recommendations according to available resources.

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Supportive care: bringing better cancer treatment – Aapro

Keywords: cancer; palliative care; quality of life; supportive care; survival

Mieux traiter le cancer grâce aux soins de soutien

Traiter selon les recommandations, même pour les soins de soutien, diminue les coûts et améliore les résultats des patients. Des projets récents accréditant les modalités thérapeutiques ont introduit de nouveaux types de toxicité ; il faudra donc de nouveaux algorithmes de traitement. Nous avons déjà constaté un résultat positif sur la survie des patients dus aux facteurs stimulant la lignée granulocytaire, aux bisphosphonates et probablement au denosumab, pour ne citer que quelques exemples. De plus, dans trois études randomisées, des soins palliatifs précoces ont eu des conséquences positives sur la mortalité due au cancer. Malgré la position de l’ESMO (European Society for Medical Oncology) prise en 2003, appelant au développement de soins palliatifs et de soutien optimaux, les patients continuent malheureusement d’être vus par ces experts tardivement au cours de leur maladie. De nos jours, la garantie de soins médicaux abordables étant un défi majeur même pour les pays possédant certaines richesses, il est possible d’imaginer que ce défi puisse être ressenti de façon plus marquée dans les pays plus limités à cet égard. Pour mieux répartir leurs ressources, ces pays pourraient adapter les recommandations existantes (comme celles prenant en charge les symptômes physiques, la douleur, la surveillance et la documentation, les aspects psychosociaux et spirituels des soins, l’éducation professionnelle de la santé, ainsi que le patient, sa famille et l’éducation des soignants) aux priorités suggérées par un groupe de consensus, comme celles rassemblées par le Breast Health Global Initiative.
The American Joint Committee on Cancer and the Union Internationale Contre le Cancer (AJCC/UICC)-TNM staging system provides the most reliable guidelines for the classification of colorectal carcinoma. This tumor staging summarizes tumor burden (T), the presence of cancer cells in draining and regional lymph nodes (N), and evidence for distant metastases (M). However, among patients within the same stage, clinical outcome can be very different. There are multiple other ways to distinguish different subtypes of colorectal cancer, including morphology, cell origin, molecular pathways, mutation status, and gene expression–based stratification. However, these parameters rely on tumor-cell characteristics. Systems biology approaches have facilitated analysis of the complex interaction between tumors and the host immune response, and allowed definition of the immune contexture. Extensive literature has demonstrated the prognostic impact of intratumoral immune cells, whose density is influenced by tumor immunogenicity, chemotraction, and adhesion. This in situ adaptive immune infiltrate can be quantified by a new methodology named “Immunoscore.” In colorectal cancer, incorporating this new methodology may add to the significance of the current classification system, as it is a prognostic factor shown to be superior to the AJCC/UICC-TNM classification. An international consortium has been initiated to validate and promote Immunoscore in routine clinical settings. Thus, Immunoscore and standardized immune parameters could become elements of the classification of cancer.

Definition of cancer

Since the launch of the US National Cancer Plan in 1971, intensive research efforts have been underway. The resulting definition of cancer seems to challenge the previously established concept of the disease. Defining cancer is fundamental, because that shapes how we view the factors influencing tumor progression, and thus, its prognosis and how we approach development of appropriate therapeutic strategies. Consequently, the importance of the tumor microenvironment and immune component of cancers has recently taken center stage.

Biological concepts that dominated the 20th century first led to a strictly cell-centric vision of cancer, giving rise to the somatic mutation theory, which defined cancer as a disease of the DNA within tumor cells. That theory held that transfer or modification of the genome gives the cell a selective advantage, in the Darwinian
sense, allowing the development of a mutated cell clone. The discovery of the Philadelphia chromosome in chronic myelogenous leukemia encouraged speculation that it might be possible to define cancer as the result of a limited number of key gene alterations. In other words, after a multistep process of acquiring successive random mutations, increasingly aggressive cell clones would be selected by the emergence of a tumor phenotype. In this cell-centric paradigm, the cancerous cell becomes autonomous, operating independently of its microenvironment. Therefore, genetic alterations were thought to dictate the clinical course of cancer, to accompany tumor progression—local, nodal, and metastatic regional—and were expected to correlate strongly with patient prognosis. Thus, it was believed that cancer treatment could aim to correct genetic alterations or to directly eliminate tumor cells.

Advances in the understanding of the molecular biology of cancer have gradually revealed the limits of the genomic cell-centric paradigm. In clinical practice, no gene or genomic signature has drastically improved prognostic classification provided by the TNM staging system (tumor, node, metastasis) in over 80 years.

**Definition of cancer integrating the tumor microenvironment**

Those observations have led to a paradigm shift where the cancer cell is no longer defined by the acquisition of key genomic alterations, but by the acquisition of key secondary behavioral characteristics through genomic changes. Six key characteristics were proposed: (i) evasion of apoptosis; (ii) self-sufficiency in growth signals; (iii) insensitivity to antiproliferative signals; (iv) stimulation of angiogenesis; (v) unlimited potential for replication; and (vi) ability to escape the site of origin and metastasize.1 Thus, at the dawn of the 21st century, the immune system was not recognized among the elements associated with cancer. However, more than a century of work by immunologists has shown the importance of that system. Beyond the now recognized essential role of the immune system in the development of cancers, a holistic vision of cancer includes the microenvironment as a real player in the development and evolution of cancer, and therefore its definition. The microenvironment is defined as a set of cellular compartments associated with cancer cells: vascular, neuroendocrine, stromal, epithelial, and immune.2 These compartments form a heterogeneous, dynamic, and communicative interaction network.

Over the years, the field of cancer immunology has endured fluctuating levels of pessimism and was often deemed controversial. The rebirth of tumor immunology really started in the early 2000s. Novel experimental observations have since invigorated the field, encompassing 3 major achievements. Firstly, immunosurveillance,3 4 the equilibrium phase of cancer,5 and immunoediting and its escape3 was shown in mouse models. Secondly, the importance of the intratumoral natural adaptive immune reaction to the survival of the patient was illustrated, showing for the first time that immune parameters come into play beyond tumor progression and invasion (TNM classification).6 7 These parameters were referred to as immune contexture (Figure 1).

Thirdly, the successes of several immunotherapies boosting this natural immune response have generated tremendous enthusiasm within the cancer immunology field.8

**Figure 1. Immune infiltrates of tumors and the immune contexture.** (A) Immune infiltrates of tumors (at the tumor center, invasive margin, and adjacent tertiary lymphoid structures) include all immune cell types. (B) The immune contexture comprises the type, density, and location of adaptive immune cells within distinct tumor regions. (Immunoscore specifically quantifies the density of memory and cytotoxic lymphocytes in the tumor center and invasive margin) and the immune functional orientation characterized by immune gene signatures.

**Abbreviations:** MDSC, myeloid-derived suppressor cells; NK, natural killer; TFH, T follicular helper cells; TLS, tertiary lymphoid structure.

**TNM classification**

The most common system for classifying the extent of the spread of cancer is the American Joint Committee on Cancer/Union Internationale Contre le Cancer (AJCC/UICC)-TNM classification.9–11 This tumor staging gives an estimation of the degree of tumor progression and invasion at the time of surgical resection. Furthermore, multiple tumor-cell parameters give an indication of the intrinsic biology of the tumor. The TNM classification has been used for over 80 years and is valuable in estimating the outcome of patients for a variety of
cancers. It is used in clinical trials to select patients who are eligible for inclusion. This powerful approach has stood the test of time for prognostication; nevertheless, it provides incomplete prognostic information. Clinical outcome can dramatically vary among patients within the same histological tumor stage. In some patients, advanced-stage cancer can remain stable for years, and in some cases, regression of metastatic tumors can occur spontaneously.

In contrast, relapse, rapid tumor progression and patient death is associated with approximately 25% of TNM I/II stage colorectal cancer (CRC) patients, despite complete surgical resection and no evidence of residual tumor or metastasis. The predictive accuracy of this traditional staging system still relies on the assumption that disease progression is largely a tumor cell–autonomous process, and fails to incorporate the effects of the host immune response. Though multiple ways to refine cancer classification have been proposed, they all rely on tumor-cell characteristics. Some examples include immunohistochemistry for tumor biomarkers, flow cytometry for DNA content, molecular signatures, or genetic features. Even imperfect, the TNM classification was never surpassed in multivariate analysis by such alternative methods.

However, we have shown that the analysis of a specific type of intratumoral immune response—by a test called Immunoscore—does indeed surpass the TNM classification in multivariate analysis. Thus, tumor progression should be considered the result of a balance between an invasive tumor process and a defense system whose major component is constituted by the host immune response.

**Selected abbreviations and acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AJCC</td>
<td>American Joint Committee on Cancer</td>
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<tr>
<td>UICC</td>
<td>Union Internationale Contre le Cancer</td>
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<tr>
<td>CRC</td>
<td>colorectal cancer</td>
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<tr>
<td>CT</td>
<td>tumor center</td>
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<tr>
<td>IM</td>
<td>invasive margin of the tumor</td>
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<tr>
<td>I0, I1, I2, I4</td>
<td>Immunoscore 0, 1, 2, 4</td>
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<td>DFS</td>
<td>disease-free survival</td>
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<tr>
<td>DSS</td>
<td>disease-specific survival</td>
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<tr>
<td>OS</td>
<td>overall survival</td>
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<tr>
<td>SITC</td>
<td>Society for Immunotherapy of Cancer</td>
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<tr>
<td>TFH</td>
<td>T follicular helper cell</td>
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<tr>
<td>TLS</td>
<td>tertiary lymphoid structures</td>
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<tr>
<td>TNM</td>
<td>tumor, node, metastasis (TNM staging system)</td>
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**Molecular subtypes of colorectal cancer**

Numerous tumor-cell characteristics have been used to classify the multiple subtypes of colorectal cancer (CRC), including morphology, molecular pathways, mutation status, cell of origin, and gene expression. A morphology-based classification allows the distinction of a number of histologic variants: mucinous, signet ring cell, medullary, micropapillary, serrated, cribriform comedo-type, adenosquamous, spindle cell, and undifferentiated.

These histopathological criteria have a modest prognostic value. CRC can also be classified by molecular pathway, eg, chromosomal instability (CIN), microsatellite instability (MSI), and a CpG island methylator phenotype (CIMP). A third method to classify CRC is based on mutation analysis, including adenomatous polyposis coli (APC), Kirsten rat sarcoma viral oncogene homolog (KRAS), tumor protein 53 (TP53), B-Raf proto-oncogene, serine/threonine kinase (BRAF), neuroblasto-toma RAS viral (v-ras) oncogene homolog (NRAS), phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α (PI3KCA), and catenin (cadherin-associated protein), beta 1, 88 kDa (CTNNB1) genes. The fourth and fifth methods, assessing the cell of origin and gene expression, are molecular-based techniques.

Numerous markers, signatures, and methods have been proposed for evaluating tumor prognosis, yet few of these translate into clinical practice or reach the statistical power of the TNM classification. Although the development of each tumor is thought of as a unique carcinogenic process, similarities may occur, ie, common pathogenic mechanisms may be involved. However, other major parameters must be taken into consideration, in particular the tumor microenvironment.
**Immunoscore as a new approach for the classification of colorectal cancer**

A potential clinical translation of the immune contexture into a prognostic marker in CRC has been established, designated Immunoscore.6,16-18 Immunoscore was initially described several years ago.7 It was shown to be a prognostic factor at baseline,14,19 but could also play a role as a marker to predict the response to biotherapies targeting the immune checkpoints.6,16,20,21 Immunoscore is based on the quantification of 2 lymphocyte populations (CD3/CD8), both at the tumor center (CT) and the tumor invasive margin (IM).14 Similar results were found when using CD3/CD45RO, or CD8/CD45RO combinations. Immunoscore provides a scoring system ranging from Immunoscore 0 (I0), where low densities of both cell types are found in both regions, to Immunoscore 4 (I4), having high immune-cell densities in both locations. Classification using Immunoscore has been shown to have a prognostic significance superior to that of the classical TNM system, for disease-free survival (DFS), disease-specific survival (DSS), and overall survival (OS). Multivariate Cox analysis revealed that the immune criteria significantly associated with prognosis for CRC stages I, II, and III.13 For the first time, tumor progression and invasion were shown to be statistically dependent on the host immune reaction, where the immune pattern remained the only significant criteria over TNM classification.13,22

CRC patients with clinically localized CRC and no detectable tumor spreading to lymph nodes or distant organs are usually treated by surgical removal of the tumor. However, approximately 25% of these patients will have recurrence of their disease, indicating that occult metastases were already present at the time of surgery. No current tumor-associated marker would predict the recurrence of this subgroup of patients, who may benefit from adjuvant therapy. In comparison, the Immunoscore approach was applied to 2 large independent cohorts, where only 4.8% of patients with I4 relapsed after 5 years and 86.2% were still alive. In contrast, 72% of patients with a low score (I0, I1, and I2) experienced tumor recurrence and only 27.5% were alive at 5 years. This illustrates the importance of Immunoscore as these I0, I1, and I2 patients potentially could have benefited from an adjuvant therapy if Immunoscore had been incorporated into the tumor staging.14

Clinical validation of Immunoscore with standardized procedures is necessary to reach clinical applicability for individual patients. The aforementioned complexity of immunohistochemistry, coupled with protocol variation, contributes to data variability. A standardized consensus method is therefore required. Large-scale assay harmonization is essential to pursue assay uniformity to reduce these limitations. In answer to this, we have performed multiple Immunoscore quality controls to evaluate the methodology for accuracy and repeatability. We observed that automated cell counting achieved a high level of correlation with optical counting for CD3 and CD8 immunostaining. In addition, the variability between users of the software was minimal.

To evaluate Immunoscore in the clinic and measure its prognostic value, we are conducting a prospective, multicenter, French national study of 600 patients from 7 hospitals. In an effort to promote the utilization of Immunoscore in routine clinical settings, we initiated a worldwide Immunoscore consortium, with the support of the Society for Immunotherapy of Cancer (SITC).21 The worldwide Immunoscore consortium, composed of international expert pathologists and immunologists, identified a strategy to show the feasibility and reproducibility of Immunoscore, validate its major prognostic power in colon cancer stage I/II/III, and to show the utility of Immunoscore to predict stage II colon cancer patients with high risk of recurrence. Evidence-based selection of specific markers for Immunoscore was discussed. The combination of 2 markers (CD3 and CD8) in 2 regions (CT and IM) was agreed for validation in standard clinical practice. Precise quantification is currently performed on whole slide sections, following the recommended initial guidelines. Twenty-three international pathology expert centers are now participating in the Immunoscore enterprise. It is hoped that this initiative will result in the implementation of Immunoscore as a new component for the classification of cancer TNM-I (Immune). Immunoscore should better define the prognosis of cancer patients, better identify patients at high risk of tumor recurrence, help to predict and stratify patients who will benefit from therapies,16 and ultimately, to help save the lives of patients with cancer.

**Immunoscore for the classification of cancer**

To be used globally in a routine manner, evaluation of a novel marker should have the following characteristics: it should be routine, feasible, simple, inexpensive, rapid, robust, reproducible, quantitative, standardized, and powerful. Immunoscore has the potential to fulfill these key criteria (Figure 3, page 338). In addition, Immunoscore provides a tool for novel therapeutic approaches, including immunotherapy.23-26 A meta-analysis summarizes the impact of immune cells including all subsets of T cells on clinical outcome from more than 120 published articles.17 Importantly, the beneficial impact of the immune infiltrate with T cells of the cytotoxic (CTLs) and memory phenotype has been demonstrated in cancers from diverse anatomical sites, including colorectal, but also melanoma, head and neck, breast, bladder, urothelial, ovarian, esophageal, prostatic, pancreatic, cervical, hepatocellular, and gastric cancers, medulloblastoma, and melanoma.17 It is interesting to note that the implications of this immune phenotype apply not only for various organs of cancer origin, but also to various cancer cell types, ie, adenocarcinoma, squamous cell carcinoma, large cell cancer, and melanoma. Thus, general characteristics emerge in which CTLs, memory T cells, and T\(1_c\) cells are associated with prolonged survival.8,16,27

Considering the probable universal character of the immune control of tumors, it is essential to take into account the immune parameter as a prognostic factor and to introduce the
Immunoscore as a component of cancer classification, not restricted to CRC (Figure 3). Accumulating evidence suggests that once human cancer becomes clinically detectable, the adaptive immune response plays a critical role in preventing tumor recurrence. The ability of effector-memory T cells to recall previously encountered antigens leads to a protective response. Following primary exposure to antigen, memory T cells disseminate and are maintained for long periods of time. The trafficking properties and the long-lasting antitumor capacity of memory T cells could result in long-term immunity in human cancer. Over the past few years, the area of immune regulation at the level of the tumor microenvironment has gained a forefront position in cancer research, in CRC, melanoma, and all other cancer types.

Monitoring of immune parameters beyond immunoscore
The analysis of 28 different types of tumor-infiltrating immune cells illustrated that the cells with the strongest impact on patient survival were adaptive immune cells. These include the cells evaluated by Immunoscore assay, T cells (CD3), CTLs (CD8), and memory T cells (CD45RO). Immunoscore represents the most powerful assay for cancer classification at baseline. Other adaptive immune cells, including T follicular helper (TFH) cells and B cells, were shown to be strongly associated with a favorable outcome and protection against tumor recurrence, whereas other cells had less impact.

Chemokines have an important role in orchestrating both innate and adaptive immune cell chemotaxis and localization within the tumor. Chemokines can direct development and maintenance of tertiary lymphoid structures (TLS), which has been described in multiple cancer types including non–small cell lung cancer, melanoma, and colorectal carcinoma. We examined the predictive capability of chemokines using data integration of gene expression in primary tumors from CRC patients. We discovered a significant prolongation of DFS in patients with high expression of the chemokines CX3CL1, CXCL10, and CXCL9. CX3CL1, also known as fractalkine, mediates T-lymphocyte and monocyte migration and promotes strong adhesion to endothelial cells. CXCL10 and CXCL9 are closely related cytokines. They facilitate migration of CTLs, monocytes, natural killer (NK), and dendritic cells. Indeed, CRC patients with elevated gene expression of one of these 3 chemokines had increased percentage and density of CD8 T lymphocytes in the tumor as assessed by flow cytometry and immunohistochemistry. High expression density of CXCL9 and CXCL10 also accurately predicts prolonged DSS in melanoma patients. Preclinical studies with melanoma show that blocking CXCL9 or CXCL10 substantially reduces the ability of CTL to traffic to the primary tumor and distant metastatic lesions. This may be due to their role in directing CTL homing to the tumor by CD4 T-cell help.

Another chemokine, CXCL13, that was recently found to be associated with TFH lymphocytes, also predicts patients’ clinical outcome. CXCL13 is produced by and has been associated with generation of TLS within the IM of primary tumors. In conjunction with this observation, CXCL13 as a single biomarker can accurately predict CRC patients’ clinical outcome. Similarly, in specific subtypes of breast cancer, elevated expression of CXCL13 in the tumor is associated with increased DFS compared with tumors with low expression of CXCL13. It is becoming increasingly clear that chemokines have an essential role in trafficking CTL to the tumor site. Furthermore the addition of chemokine expression to Immunoscore has potential to predict patient response to chemotherapy.

Correlating immune responses and clinical outcome in immunotherapy trials
The efficacy of immunotherapeutic interventions is generally assessed by parameters defining the clinical outcome, such as OS or PFS. Clearly, immune responses can be assessed much earlier than these clinical parameters and thus be used for immunoguiding purposes during therapy, or to stratify patients prior to treatment for maximum benefit. Various clinically successful immunotherapeutic approaches have been reported, such as sipuleucel-T for prostate cancer and ip-
Immunoscore in human tumors – Galon

Indeed, innovative therapies that induce longer survival in patients modify the immune contexture of the tumors, usually by inducing an increased infiltration of CD8+ T cells.17 It is the case for classical chemo-49 and radiotherapies, for targeted therapies such as Braf inhibitors,50 or inhibitors of angiogenesis that decrease regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs),51,52 and of course for immunomodulatory antibodies such as anti–CTLA-4,53 anti–programmed cell death protein 1 (anti–PD-1)23 and anti–programmed death-ligand 1 (anti–PD-L1).54,55

Conclusion

Given the power of immune-cell–infiltration quantification, it is hoped that Immunoscore will become a new component for the classification of cancer, leading to a TNM-I (Immune) classification.56 Immunoscore should better classify cancer patients at baseline, better define the prognosis of cancer patients, better identify patients at high risk of tumor recurrence, help to predict and stratify patients who will benefit from therapies and, ultimately, to help save the lives of patients with cancer.

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References


**Keywords:** adaptive immunity; cancer classification; immune contexture; Immunoscore; immunotherapy; T cell; tumor microenvironment

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**L’IMMUNOSCORE, UNE NOUVELLE APPROCHE ÉVENTUELLE DE LA CLASSIFICATION DES CANCERS.**

The « macro » of stars and the « micro » of cells occasionally share uncanny resemblances, as shown on this page. Today’s science is entering new, seemingly unbounded spaces where state-of-the-art imaging technologies reign supreme. The fascinating and strangely beautiful images above tell us stories: about the mysteries of our origins, somewhere in the fathomless primeval Universe (or universes?), and the mysteries of our very being, reaching into the core of our cells and elucidating the metabolic processes that spell out life or disease. Two of France’s leading scientists explore these new worlds for us.

**FROM MACRO:**

The Universe has a history, but did it ever have a beginning?

Étienne Klein, France

Page 343

**TO MICRO:**

Innovative biological technologies: cracking the cell’s ultimate secrets?

Spencer Shorte, France

Page 353

*Top: ESO 149-3 galaxy 200 million light years from earth. © Science Photo Library/Cosmos*

*Bottom: Primary human macrophage infected by HIV pseudo virus with luciferase gene. © Institut Pasteur/Asier Saez-Cibion, unité Régulations des Infections Rétrovirales - Marie-Anne Nicola, Plate-Forme d’Imagerie Dynamique*
Echoes of the early Universe. False-color microwave map of a section of the whole sky, showing variations in the cosmic microwave background. The mean temperature of the background radiation (2.73 kelvins) is shown as deep blue; pink and red areas are warmer, while blue areas are cooler—red is +0.27 millikelvins (mK), pale blue is −0.27 mK. Computer analysis of the image shows that the pattern of fluctuations in the background is consistent with the inflationary Big Bang theory of the creation of the Universe, and the existence of invisible dark matter. Data obtained by the Differential Microwave Radiometer (DMR) on the Cosmic Background Explorer (COBE) satellite over 2 years. © NASA/Science Photo Library/Cosmos.
The Big Bang theory, formulated in 1927 by the Belgian Catholic priest and astronomer Georges Lemaître, was confirmed in 1964, thanks to the discovery of the “cosmic background radiation.” The scientific community finally recognized the evidence that the Universe is indeed expanding and that its temperature has necessarily dropped over time. The very existence of this background radiation indicates that in its distant past the Universe inevitably passed through a much denser and hotter phase.

Here I retrace the story of how scientists dated the universe to 13.7 billion years ago, to the ultradense and ultrahot phase that has come to be known as the Big Bang. Unlike its image in the popular imagination, this Big Bang is not the primordial explosion thought to have created everything in existence. Can we describe this time zero, think about it, tell where it came from? Where did the pre–Big Bang universe come from? Mystery! So, whether or not the Universe had an origin, we never start from zero. Discussing it means talking about something that was already there. And yet if something was already there, we are not talking about the origin of the Universe, but rather about a stage in its history. Is the question of a beginning in fact endless?

E  tienne Klein holds PhDs in the philosophy of science and in physics, and is director of research at the French Atomic Energy Commission, where he heads the Materials Science Research Laboratory (LARSIM). He has taken part in many large projects, notably the development of atomic vapor laser isotope separation and the study of superconducting cavities for particle accelerators. At CERN (European Organization for Nuclear Research), he was involved in the design of the Large Hadron Collider (LHC), which was used to find the Higgs boson in July 2012. Professor of physics and of the philosophy of science at the École Centrale in Paris, Étienne Klein is the author of numerous books, the recipient of many prizes, and a member of the French Academy of Technologies. He presents “Scientific Conversation,” a weekly radio program, on France-Culture.

**History prides itself on speaking only of memory, but in reality often forgets. It forgets, for instance, what happened before the appearance of humans, just as it overlooks the past of inert things like atoms and rocks, stars and galaxies. History covers but a few millennia, whereas the Universe began (if it did indeed have a beginning) at least 13.7 billion years ago. This scientists were able to establish during the 20th century, by dating other beginnings, some very ancient, at the heart of the Universe itself. The Earth was formed 4.45 billion years ago, life appeared close to one billion years later, and humans emerged a little under 2 million years before the present. Yet what in truth do these numbers tell us? They tell us that in its past the Universe contained objects much older than any life form on Earth and that countless events followed one upon another unseen by human eyes. Humankind, a recent species when all is said and done, was not there to witness...**
the myriad events that mark the unfolding story of the Universe. Far from it. Two million years versus 13.7 billion is a ratio of 1 to 6850! In other words, if the duration of the Universe is likened to 24 hours, we humans appeared on the scene at 12 seconds to midnight. We must resign ourselves to the fact that the Universe has spent the better part of its time doing without us.

On our curves, graphs, or diagrams, we always show the timeline as straight, its direction indicated by a small arrow. A straight line is, by definition, infinite. But is a timeline infinite?

In other words, does the timeline stretch unbroken into the past and the future? Has there always been time and will there be forevermore? Is the timeline not instead a half-line, with an origin or first point, a first instant? And if so, can we conceive of this “time zero?” Are we able to describe it, to speak of it, to relate from whence it came? Philosophers, metaphysicians, and theologians have long argued fervently about these questions. Have men and women of science nothing to add? Of course they do—up to a point.

The idea of Universe
Today we know that the Universe is not static, that it can even be read like a long, unfolding story. And this truth is the outcome of an extraordinary adventure in the realm of ideas that went on for centuries until, in the 1930s, it suddenly acquired new meaning and, above all, unprecedented import. The Universe then has a history, but before speaking of a putative origin, we need to understand what we mean by “the Universe,” and for that we have to bear three things in mind.

First, the meaning of the word Universe has changed over the ages, according to how it was portrayed or imagined. Today, the Universe is no longer depicted as resting on a pile of tortoises or whales, as affirmed in certain cosmogonies. It is not reduced to the solar system or to the “cosmos” of the Ancient World, or to some vague envelope containing everything there is. The idea of a “Universe” in the scientific sense is a recent invention, which we owe to Galileo: comprising a single “matter,” the Universe is governed by “universal” laws expressed in mathematical form that are the same everywhere and at all times.

Andromeda galaxy (M31), optical image. The galaxy comprises a central nucleus surrounded by spiral arms. The nucleus appears more yellow than the spiral arms as it contains a higher proportion of dust and older, redder stars. Two small satellite galaxies are associated with Andromeda, M32 (NGC 221), the fuzzy blob at lower right, and M110 (NGC 205), the bright star-like point immediately left of Andromeda’s nucleus. The Andromeda galaxy is the closest major galaxy to our own Milky Way, lying just over two million light-years away. © Robert Gendler/Science Photo Library/Cosmos.
Second, the gamble of considering the Universe as a subject of scientific inquiry, characterized by inherent and measurable parameters, dates from barely a century ago. The scientific idea of the Universe formulated by Galileo and reworked by Newton, who drew up the first “universal” theory (of gravity), therefore did not suffice as a subject of scientific inquiry like (almost) any other. Because it does not follow that the container of all physical objects is itself one. To make this conceptual leap a new, literally revolutionary theory was needed—Einstein’s general relativity in 1915—which apprehended the Universe in its entirety and not solely by means of the physical objects of which it is the vast receptacle.

Third, asserting that the world’s objects have a history, that the world does too, or that there are histories in this world, does not mean that the Universe itself has one. The idea that histories unfolded within the cosmos is doubtless as old as all the first “histories of the world.” Moreover, what would a history of the world be that didn’t relate histories in and of this world? These histories though relate only to what happens in the Universe and not to the Universe as such. Only during the 1930s were physicists able to establish that the Universe is expanding and that it too therefore has its own history.

The Universe has a history

It was the twofold thrust of science and technology that wrought this understanding of the Universe and showed that it is expanding. Jacques Merleau-Ponty neatly summarized it thus: “a physicist of genius and a giant telescope, operated by a practiced astronomer, each added something to the philosophy of Nature—one an idea, the other a vision of the Universe—without it being possible to say which of the two was more surprising and thrilling.” The “physicist of genius” was, of course, Einstein, who in 1915 formulated a new theory of gravity: general relativity theory. Newton conceived of gravity as an instantaneous attractive force acting over a distance between two massive objects. Einstein understood things differently. He saw gravity not as a force acting through space, but rather as the effect of a deformation that matter imprints on space-time. Far from being static and rigid, space-time appears to be flexible and dynamic: it can, for instance, curve, dilate, or contract.

To grasp the meaning of Einstein’s theory, imagine a bed sheet stretched taut with a baseball in the middle. If the sheet is shaken gently, bumps and hollows appear in its surface and these deformations force the ball to move—down slopes at pace and up slopes more slowly. It is the shape of the sheet’s surface, its “geometry,” that dictates the ball’s path. Yet the ball is not a purely passive object—its weight and movement alter the shape of the sheet. The very presence of the ball, for example, would perturb the trajectory of a ping pong ball rolled in a straight line, as would someone shaking the sheet.
Big Bang, conceptual image. Computer illustration representing the origin of the Universe. The term Big Bang describes the initial expansion of all the matter in the Universe from an infinitely compact state 13.7 billion years ago. The initial conditions are not known, but less than 1 second after the beginning, temperatures were trillions of degrees Celsius and the primordial Universe was much smaller than an atom. It has been expanding and cooling ever since. Matter formed and coalesced into the galaxies, which are observed to be moving away from each other. Background radiation in the Universe is considered a remnant of the Big Bang.

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As for Merleau-Ponty’s astronomer with his giant telescope, he is Edwin Hubble, at the Mount Wilson Observatory. In 1929, a few years after Einstein announced his theory, Hubble discovered the law that bears his name. It states that galaxies are moving away from each other at a speed that increases as they move farther apart. In reality, it is not the galaxies that are speeding apart through space, but space itself that is expanding, bearing the galaxies with it. So the Universe is not static: it is expanding.

Knowing this, what if we were to run the cosmic film backwards? We would see that, in its distant past, the Universe was much smaller and denser than today. And being denser, it was also hotter. And if, using Einstein’s equations, we extrapolate this situation as far as possible into the past, we end up with a Universe of zero size, an origin, a “Big Bang” characterized by infinite temperature and density.

Einstein and Hubble were therefore pioneers of a new science: scientific cosmology. We should, however, also mention the contributions of the Russian Alexander Friedmann and the Belgian Georges Lemaître.
In a 1922 article on the curvature of space, Alexander Friedmann showed that Einstein’s general relativity equations have all kinds of solutions corresponding to universes that are not static: the space they enclose varies as a function of time. This opened minds to the idea that the Einsteinian conception of gravity allows the existence of nonstationary universes, i.e., universes with a history and therefore perhaps an origin.

As for Georges Lemaître, he was the first to hypothesize, in 1927, before Hubble made his observations, that the Universe is expanding. From the early 1930s, Lemaître set out his hypothesis of “the primeval atom,” which foreshadowed the idea of the Big Bang and, at the time, left his colleagues unconvinced.

The strictly physical consequences of the expansion of the Universe discovered by Hubble, to wit that the contents of the Universe should also evolve, were not accepted immediately. It was only in 1964, thanks to the discovery of a very particular radiation—the “cosmic background radiation”—that the scientific community finally recognized the evidence that the Universe is indeed expanding and that its temperature has necessarily dropped over time. The very existence of this background radiation indicates that in its distant past the Universe inevitably passed through a much denser and hotter phase. What does this mean? During the first 380,000 years after the Big Bang light was present everywhere in the Universe, but could not move freely through space. The small packets that make up light, which are called photons, could not budge without immediately colliding with other particles, like electrons or protons. Matter thus hindered the propagation of light. But as the Universe gained in size, its temperature fell. And when the temperature reached 3000 kelvins (i.e., 2727 degrees Celsius), the electrons were able to combine with nuclei to form atoms. Since the photons interacted little with the atoms, they were at last able to propagate freely in the Universe. It is the light the photons form today, the light suddenly released from matter after 380,000 years of imprisonment, which forms the cosmic background radiation. In a way it is the trace left today by the very hot phase through which the Universe passed long ago, when still very young.

Cosmology today is a well-established science. Thanks to constant improvements in their instruments, physicists are gaining an ever-increasing understanding of the properties

The aftermath of the death of a massive star is shown in detail in this composite image of G292.0+1.8. In color is the Chandra X-ray Observatory image and in white are optical data from the Digitized Sky Survey. Near the center of G292.0+1.8 is the so-called pulsar wind nebula, most easily seen in high energy x-rays. This is the magnetized bubble of high-energy particles that surrounds the “pulsar,” a rapidly rotating neutron star that remained behind after the original, massive star exploded. The narrow, jet-like feature running from north to south in the image is likely parallel to the spin axis of the pulsar. Each color represents different elements such as oxygen, neon, magnesium, and silicon. The distance estimated of this object is about 20,000 light-years. © NASA/CXC/Novapix.
of the Universe. They have, notably, obtained precise information on its shape, large-scale structure, and evolution. Furthermore, they know how atoms were formed during the history of the universe.

Where do atoms come from?
The water we drink, even when we call it “fresh,” does not come from the latest rainfall. Whatever its source, water is never “from the fountain of youth.” A water molecule comprises two hydrogen atoms and one oxygen atom. Hydrogen atoms were formed in the primordial Universe (13.7 billion years ago) and oxygen atoms in the heart of a star (some 5 billion years ago), which then scattered them through intergalactic space. Quenching one’s thirst is therefore a serious and profound act that ties us to practically the whole history of the Universe. When we drink water, we are in fact imbibing the remnants of the dawn of the Earth mixed with ash from later starbursts.

How did we learn this? Astrophysicists and nuclear physicists have been able to piece together the saga that leads back to the primordial Universe, all the way to the entities that constitute today’s matter. We could begin by saying that a few minutes after the Big Bang the Universe was still very hot (the word is scarcely adequate as we are talking of a temperature of around a billion degrees Celsius). This suggests that we are certain that there was a beginning, marked by a time zero, which we have been able to fathom. Time zero has not withstood the scrutiny of quantum physics: it has skedaddled goodness knows where, leaving no news and no forwarding address. It is therefore best to say that when the temperature was about one billion degrees Celsius and the density was comparable to that of ambient air, the Universe was a sort of huge cosmic cauldron able to generate scraps of material structures, albeit cooling as it expanded. Here there were protons, neutrons, electrons, and photons, moving every which way and constantly bouncing off each other. The photons, whose energy until then had always been enough to overcome the force holding protons and neutrons together, ended up by being too “soft” to break them apart: the nuclei of deuterium, assemblies of one proton and one neutron, were then able to start forming. Once they appeared, these deuterium nuclei fused in pairs or in turn captured a proton to form helium nuclei.
Pairings of this sort generally happened fast, but some protons remained alone and later served as nuclei of hydrogen, the lightest chemical element. These pairings were not always lasting. There were brief affairs or simple encounters with no tomorrow: nuclei that survived for extremely short times. Victims of their instability, they quickly split into other lighter nuclei while emitting radiation: they were radioactive.

After just three minutes of this little game—collisions, pairings, break-ups—the Universe contained nuclei of hydrogen, deuterium, helium, lithium, and beryllium. But nothing else: no carbon, no oxygen, no heavy nuclei. The move towards complexity was suddenly blocked. There’s a reason for this: the Universe was already so diluted by its expansion that the nuclei and their constituent components (nucleons) were far from one another and no longer able to meet and form bigger nuclei. No more encounters, so no more pairings.

Things obviously did not end there. Much later, the birth of the stars enabled the formation of heavier elements, from carbon to iron and to uranium, progressively synthesized through a succession of nuclear reactions, within the stars themselves or in explosions of massive stars. In all its phases (primordial, stellar, or explosive), nucleosynthesis—the creation of new nuclei from nucleons—started with the basic ingredients, protons and neutrons, which it formed into increasingly heavy nuclei. The appearance of chemical elements in the universe is therefore in no way a creation ex nihilo. On the contrary, it is the culmination of the processes that produced the elements. The “origin” of the elements is explained by describing how what came before could have generated them, by explicating the successive physical phenomena that led to them. The question then is where did the protons and neutrons come from? Particle physicists have now answered this question: from the combination of quarks (in groups of three) and gluons in the primordial Universe. But where did these elementary particles, the quarks and gluons, which have no known internal structure, come from? No one has yet answered this question: quarks and gluons have no identified origin. If they were born, their progenitors remain nameless.

**What’s in a name? The Big Bang**

Since we now know that the Universe is not static, that it has had and continues to have a history, we tend to believe that this history inevitably had a beginning. But are we right? Strictly speaking, the Big Bang designates the very dense and hot epoch of the Universe 13.7 billion years ago. But in general it is used with a very different meaning: to denote the original explosion that is believed to have created everything that exists, in other words the time zero marking the simultaneous and sudden appearance of space, time, matter, and energy. In common parlance, the Big Bang has come to mean the very creation of the world, a physical equivalent of the Biblical “Let there be light.”

In theory, this is not a misinterpretation. Delving ever further into the past of the Universe, we discover that the galaxies move nearer to each other and, if the equations of general relativity are to be believed, that the Universe shrinks to a point, ie, to zero volume. In other words, if we run the clock backwards, calculations show that 13.7 billion years ago there was a time zero directly associated with what physicists call an “initial singularity”: a situation in which temperature and density become infinite. So what prevents us from likening this initial singularity to the effective origin of the Universe? On the face of it nothing, until we look a little closer.

While our way of speaking of the Big Bang has scarcely changed since 1950, the year it was christened and started to be popularized, many things have since happened in the fields of astrophysics and cosmology, to the point that there is a need to change our way of viewing the Big Bang and how we speak about it.
What have we understood that is new? In the 1950s, the description of the Universe was rooted exclusively in the equations of general relativity, which, as we have seen, describe the effects of gravity. Yet when we run time backwards, the Universe shrinks and so matter ends up in very special physical conditions that general relativity alone is unable to describe, because forces other than gravity come into play.

When temperature and density become extremely high, the behavior of particles of matter is determined by the electromagnetic force, the strong nuclear force, and the weak nuclear force. The electromagnetic force ensures the cohesion of atoms and molecules and governs all chemical reactions as well as optical phenomena. The weak nuclear interaction is notably responsible for beta radioactivity, by which a neutron disintegrates into a proton and an electron, with simultaneous emission of an antineutrino. And the strong nuclear force holds protons and neutrons together within atomic nuclei.

General relativity takes none of these three forces into account, so physicists have understood that it alone cannot describe the first instants of the Universe. Its equations break down when the hugely energetic particles present in the Universe interact with forces other than gravity.

To peer into and speak of the conditions of the primordial Universe, physicists must enter what is called the Planck Epoch (named after the German theoretical physicist Max Planck), which covers the first 5.4x10^-43 seconds at the beginning of the universe. This happened 13.7 billion years ago and present-day physics cannot describe what occurred during this instant in time. So we are unable to run the history of the Universe back to its origin, if origin there was. We have reached the limit of applicability of our current concepts of physics. Beyond this point, our physics is no longer valid.

Origin or no origin?
How then can we come up with a better, and above all complete, description of the ultrahot and ultradense phase that is the primordial Universe? Theoreticians have posited a whole range of hypotheses: space-time has more than four dimensions (six additional space dimensions, in fact); on a minuscule scale space-time is discontinuous rather than smooth, i.e., it comprises small grains; space-time is theoretically derivable or deducible from something more fundamental, which is not space-time. What’s important is that all these theories have the property of questioning the very existence of time zero. Applied to the remotest phases in the history of the Universe, the calculations no longer indicate an "initial singularity." So, no more time zero! Everything happens as if these theories abolish, or at least sideline, the origin of the Universe.

In any case, no theory gives substance to the idea of a creation from nothing, so we are forced to review our way of thinking about the Big Bang. For instance, some theoretical models interpret the Big Bang not as a singularity, but as an extremely dense phase that may have served as a "bridge" between our expanding Universe and another universe that preceded it (the same, but contracting). In such a case, the Big Bang can no longer be conflated with the origin of the Universe. The question therefore remains open. No one is in a position to demonstrate scientifically that the Universe did or did not have an origin. If origin there was, which science has yet to apprehend, the Universe emerged from nothingness, an emergence no doubt indescribable (because to explain how nothingness ceased to be nothingness, we have to ascribe to it properties that, by their very existence, distance it and distinguish it from itself). If, on the other hand, the Universe had no origin, nothingness never existed—something was always there—in which case the question of the origin of the Universe was just an old problem ill-posed.

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L’Univers a une histoire, mais a-t-il jamais commencé ?
Nous racontons comment les scientifiques sont parvenus à remonter l’histoire de l’Univers à 13,7 milliards d’années, jusqu’à cette phase très dense et très chaude qu’on a appelée le Big Bang. Mais celui-ci n’est pas, comme on l’a imaginé, cette explosion originelle qui aurait créé tout ce qui existe. Cet instant zéro, peut-on le décrire, le penser, raconter d’où il peut bien provenir ? Et d’où serait venu l’Univers d’avant le Big Bang ? Mystère ! Qu’il y ait origine ou pas, on ne part donc jamais de zéro. En parler, c’est parler de quelque chose qui était déjà là. Or, si quelque chose était déjà là, c’est bien qu’on ne parle pas de l’origine de l’Univers, mais d’une étape de son histoire. Le début serait-il une question sans fin ?

The Universe has a history, but did it ever have a beginning? – Klein
As in the time of Hooke and Leeuwenhoek, new and emerging bioimaging technologies continue to open up new avenues of discovery and experimental investigation. Indeed, these great scientists would probably be aghast at the incredible cell imaging microscope technologies available to us today. At the time of writing, it is significant that no fewer than six Nobel Prize in Chemistry laureates in as many years have been honored for contributions that directly enhance biological imaging microscopy.

The journey between macroscopic, microscopic, and nanoscopic scales in biomedicine can be uniquely appreciated from a historical perspective looking at the development of microscopy during the last 300 years. From this history a plethora of ever more sophisticated and powerful “imaging technologies” have emerged, which have made it possible to visualize biological organic matter, from cells to whole organisms, with unprecedented spatial and temporal resolution. This article presents a synopsis of this history, accompanied by some modern examples of the current state of the art. The contributions of innovative biological imaging technologies have been recompensed by six Nobel Laureates in so many years. Technological advances in biological imaging are opening up new research possibilities like never before and look set to take central stage in terms of their usefulness for life sciences and public health.

Optics at the beginning of enlightenment

It is more than coincidence that the beginning of our journey from the macroscopic to the microscopic world began with the birth of modern science, physics, and optics itself, somewhere around 1000 AD. Before the last millennium there had been no tools to help better see the natural world surrounding us, and telescopes and microscopes were not even a figment of the popular imagination. Indeed, not even reading spectacles were invented before the 13th/14th century. Nonetheless,

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Profesor Spencer Shorte graduated (1987) in Biochemistry from the University of Kent at Canterbury (UK) and received his PhD (1992) in the same subject from Bristol University (UK). He worked developing live cell microscope imaging through several postdoctoral fellowships in Europe and eventually was appointed (1998) Assistant Professor in the USA (Medical University of South Carolina). He joined faculty at the Institut Pasteur in Paris in 2001 where he established an internationally leading imaging center (Pasteur-Imagopole, www.imagopole.org) focused on cutting-edge technologies for live cell studies. Shorte’s work developing dynamic cell and tissue imaging techniques at Pasteur brought him to become expert in the advanced imaging technologies targeting experimental paradigms relating to fundamental cell biology, immunology, and infection. He is author of more than fifty research articles, learned reviews, and five patents; his work on microrotation tomography imaging, developed in collaboration with the French mathematician Professor Bernard Chalmond (École Normale Supérieure) received the French Engineer of the Year Award 2005 in the category of science.
State-of-the-art cell imaging technologies. Mitochondria in human colorectal adenocarcinoma. 3-Dimensional fluorescence microscopy reconstruction. Nuclei are stained in Hoechst blue, mitochondria in green, F-actin network cytoskeleton in red. Blood cells are autofluorescent. © Institut Pasteur/Laurent Chatre, Unité de Génétique Moléculaire des Levures en collaboration avec Prof. Fabrice Chrétien, Unité d’Histopathologie Humaine et Modèles Animaux.
from these humble beginnings upon the “shoulders of giants” during the next several hundred years came a scientific renaissance. Sometime beginning the late 16th to early 17th centuries, setting the trend for the future, it was literally the white light of technological innovation that drove scientific discovery founded, a priori, upon impetus provided by the invention of the telescope and the microscope. These novel devices were based on the use of optics honed by artisans, essentially glass-grinding skills, and producing lenses for magnification of objects both near and far. From the invention of optical magnification was to emerge the most profound shift in the way we perceive and study the tangible Universe. The awakening of the scientific consciousness initiated toward the end of the first millennium by Moorish scholars such as Ibn Al Haytham was to be adsorbed into the European Renaissance, generating a technological revolution that would grow to challenge even the most vivid imaginations of the fantastic. During the 16th to 19th centuries, many scientific pioneers would contribute to establishing beyond a shadow of a doubt the existence of the microscopic world. These early pioneers had the burden of proving the reality of the unseen to a public audience for whom disease arose spontaneously from demonic spirits haunting dead matter. In 1665, it was the publication of the seminal document *Micrographia* by Robert Hooke (1635-1703) that described the first insights into the microscopic world that opened a new era of change.

The discovery of the microscopic scale world

While the invention of the telescope translated into immediate value for astronomy and navigation, that of the microscope, on the other hand, had found little practical application. *Micrographia* presented for the first time a systematic approach to microscopic scale observations, recording, annotating, thereby documenting a scientific view of the emerging microscopic world. *Micrographia* captured the popular imagination, and though Robert Hooke had been derided and mocked by the popular press, he was celebrated among London’s intellectual circles, including great names such as Samuel Pepys (1633-1703) who marveled at his findings. *Micrographia* became known as the first reference to the term “cells” as it is used today in modern biology, in Hooke’s case to describe the tiny repeating structures he observed in slices of cork—indeed dead cells.

One of the most magnificent plate drawings in *Micrographia* is of the common flea. Hooke certainly had no idea that the insect he observed was a central player in the *Plague of Black Death* that besieged the City of London while he worked. Black rats infested the City of London at that time and the flea, easily moving from rodent to humans, was responsible for the spread of the bacteria *Yersinia pestis* that caused millions of deaths across Europe between 1100 and 1600. A year after the publication of *Micrographia*, the Great Fire of London (1666), quite fortuitously, destroyed the pestilence, which did not return. This somewhat ironic anecdote explains why the appeal of Hooke’s observations remained largely aesthetic, and nobody suspected at that time that there was a further unseen world inside the flea, let alone one that could cause such devastating disease.

The discovery of microorganisms

It is said that a copy of *Micrographia* eventually found its way into the hands of Antonie van Leeuwenhoek (1632-1723) and inspired him his critical contribution that brought many to refer to him as the “Grandfather of Microbiology.” Leeuwenhoek took microscopy to another level by developing an effective and cheap method for producing high-quality lenses allowing some 200x magnification, much higher than previously possible. With this device, which was deceptively simple, Leeuwenhoek glimpsed for the first time the living microscopic world in the form of live bacteria, which he reported to...
the entire scientific satisfaction of the Royal Society in 1673. The Royal Society in London went on to publish hundreds of Leeuwenhoek’s letters describing his fascinating observations. He used his microscope to probe everything from pond water to semen and the plaque between his teeth. He is therefore rightly considered to be the scientist who discovered bacteria, sperm, and far more. However, his observations remained confined to the category of “curiosities.” Leeuwenhoek was not a scientist (he was a merchant by trade), so while he persevered with the discovery of what he called “animalcules,” he was ill-equipped to draw any experimentally driven scientific conclusions. Consequently, the significance of his discoveries remained hidden, and the general scientific community continued to adhere to the Aristotelian dogma of “spontaneous generation” as the basis for the origin of life. Without a scientific champion capable of proving through experimentation the significance of microscopic organisms, Leeuwenhoek’s discoveries could not be linked to any macroscopic reality, any function, or biological consequence. In fact, it was not before another 200 years that formal indisputable experimental demonstration would show that underlying the macroscopic world in which infectious disease was rife, Leeuwenhoek’s microscopic world was the origin of propagative living organisms, of pathogens capable of infesting their hosts and causing all manner of devastating infectious diseases.

**Germ theory: the causal link between “macro and micro”**

It was *The Origin of Species* published by Darwin in 1859 that fueled the debate over spontaneous generation and drove Louis Pasteur (1822-1895) to carry out a series of experiments between 1860-1864 that showed definitively that “spontaneous generation” does not occur. His experiments settled once and for all not only a philosophical problem, proving that life is not spontaneously generated from dead matter, but also served to establish the new science of microbiology. Pasteur’s landmark work debunking spontaneous generation established the “germ theory” according to which infectious diseases are caused by microorganisms too small to see without magnification, known today as virus, bacterium, protist, fungus, and prion.

The germ theory had been proposed as early as the mid-16th century and certainly gained widespread credence when substantiated by scientific discoveries of the 17th through the late 19th century, including Leeuwenhoek’s observations. But it was Pasteur who formalized experimental microbiology, along with Robert Koch (1843-1910). In scientific and philosophical terms, the work of Pasteur and Koch at the end of the 1900s linked for the first time the unseen microscopic world with the unarguably tangible macroscopic reality of infectious disease.
Modern microscopy and cell imaging

As in the time of Hooke and Leeuwenhoek, new and emerging bioimaging technologies continue to open up new avenues of discovery and experimental investigation. Indeed, these great scientists would probably be aghast at the incredible cell imaging microscope technologies available to us today. At the time of writing, it is significant that no fewer than six Nobel Prize in Chemistry laureates in as many years have been honored for contributions that directly enhance biological imaging microscopy.

The 2008 prize shared by Osamu Shimomura, Martin Chalfie, and Roger Y. Tsien was awarded for the discovery and development of genetically encoded green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*. This work transformed the power of fluorescent optical microscopy by introducing a truly postgenomic tool, whereby cells in any organism can be specifically targeted with fluorescent protein label by genetic engineering. For example, GFP can be targeted to a specific protein like H2B, a histone-binding protein abundant in eukaryotic nuclei. Transgenic expression of

**Use of green fluorescent protein (GFP) to track early embryonic cell divisions in Caenorhabditis elegans nematode worm.** Phase contrast images showing (top panel) the adult nematode worm, and (middle panel) early embryonic divisions 1-4 cell stages (left to right). **Bottom panel:** GFP fluorescence recorded from the living *C. elegans* expressing H2B-GFP nuclear histone binding protein abundant in eukaryotic nuclei. Each image from left to right shows 2-cell, 4-cell, and 64-cell stages of embryogenesis. Fluorescent images were recorded as a part of the study published in reference 7 (experiments performed by Jean-Yves Tinevez).

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**Aequorea victoria jellyfish (top panel).** Fluorescence photograph. The fluorescence comes from a green fluorescent protein (GFP) that has proved to be an incredibly valuable tool as a marker in biotechnology. The first discovery of GFP was by Osamu Shimomura in these jellyfish at the Friday Harbor Marine Laboratory. Photograph taken at the New England Aquarium.

© Charles Mazel/Visuals Unlimited/Corbis.

**Shigella flexneri, causative agent of bacillary dysentery.** The image series here shows visual analysis of *S. flexneri* infection in guinea pig colon recorded as a part of a research study. The virulent strain of *S. flexneri* used was transgenically modified to express GFP so that it could be detected by fluorescence microscopy (middle panel, right) and localized relative to colonic tissue cell nuclei (middle panel, left) and cell microfilaments (lower panel, left). The separate channels revealing the distinct elements are merged in the (lower panel, right) bacteria (green), nuclei (blue), and actin cytoskeleton microfilaments (red). Experiments designed and performed by Ellen Arena, Jean-Yves Tinevez & Philippe Sansonetti, Unité de Pathogénie Microbienne Moléculaire, Institut Pasteur.

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the chimeric fusion molecule H2B-GFP can be monitored using fluorescence microscopy directly inside a live organism model as shown for the soil nematode Caenorhabditis elegans. By virtue of H2B-GFP, a transgenic C. elegans can be monitored by fluorescence microscopy during early embryogenesis, revealing the endogenous H2B-GFP–positive cell nuclei, their spatial disposition, and temporal dynamics during cell division.1 Bacteria in a slice of gut tissue prepared from a mouse model infected with a virulent strain of infectious Shigella genetically modified to carry the GFP can be tracked and localized visually. There are literally hundreds of thousands of published reports using GFP for transgenic manipulation of cells, tissues, and even entire organisms, from microorganisms, to insects, fish, and mammals.8,10,16-19 Further, there is a plethora of modified variants characterized by different fluorescent colors and functional characteristics like, for example, environmental sensitivity to pH, light, binding with specific endogenous signal transduction proteins, ions, or metabolites.8,10,16-19 Thus, the relatively simple idea of transferring a fluorescent protein from this otherwise rather humble marine creature fundamentally changed the way we do biology. It began a revolution that continues today, and certainly provided the impetus for the more recently shared Nobel Prize in Chemistry laureates Eric Betzig, Stefan Hell, and William Moerner designing and performing the PALM/STORM method.

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The simian kidney fibroblast fluorescence microscopy images featured on this page show two different examples of SR performed in our own laboratories. The first collectively referred to as photo-activated localization microscopy (PALM) or stochastic optical reconstruction microscopy (STORM).46,49-51 We use fluorescent molecules that can be targeted to a specific structure, and upon illumination can be switched on or off, or from one color to another, normally by light control. As the individual single molecules light up uniquely at different times, they allow a highly precise estimate of the x,y spatial coordinates of the molecule in question. This blinking cycle recorded by hundreds, if not thousands of images, slowly reveals with high spatial resolution precision where exactly each individual molecule resides in a field of view. Consequently, mathematical reconstruction allows nanoscale structures to be extrapolated with far greater resolution than conventional optical imaging. The image shows a chemically fixed COS-7 simian kidney fibroblast expressing photoswitchable fluorescent protein mEos chimerically tagged to the abundant cytoskeletal protein actin.46,49-51 Top left panel: cycles of 390 nm low level illumination light cause stochastic activation of single molecules whereby the otherwise green fluorescent emission peak at 516 nm converts to red emission at 581 nm. The red emission is detected in a series of hundreds to thousands of images like the one shown, resembling a star-filled night sky rather than a specific biological structure, but the x,y coordinates of each activated molecule can be estimated with extremely high precision compared with conventional optical microscopy. Top right panel: mathematical reconstruction from the series of photoactivated mEos-actin images collected during the many repeat cycles of single molecule photoactivation. Lower panels: comparison of image quality detected in the same sample region of interest visualized using standard confocal fluorescent microscopy (lower left panel) with the PALM/STORM superresolution method (lower right panel). Experiments designed and performed by Audrey Salles, Plateforme d’imagerie dynamique, Imagopole, Institut Pasteur.
kidney fibroblast expressing a photoswitchable fluorescent protein mEos chimerically tagged to the abundant cytoskeletal protein actin. The Eos protein is originally isolated from the coral Lobophyllia hemprichii and modified for optimization in mammalian cells.46,49-51 The protein undergoes an irreversible phototransformation in response to 390 nm illumination, where- by its otherwise green emission peak at 516 nm is converted to a red emission peak at 581 nm. Thus, through repeat cycles of weak 390 nm illumination just sufficient to activate a subpopulation of mEos-actin molecules in a field of view, some molecules are red photo-converted, allowing PALM to be performed. The results in the lower panels compare the resolution detected by a standard confocal fluorescent microscope with the PALM method. For comparison, a second example of an SR-modality called structured illumination microscopy (SIM) does not depend upon any special photoactivation properties of the fluorescent molecules used, and is therefore more flexible. SIM-SR methods52,53 exploit illumination by high spatial frequencies introduced during excitation using fine gratings that can project sinusoidal illumination patterns into the sample volume. This augments the contrast of the excitation structure to be maximal, allowing high-resolution images to be reconstructed from any series of images recorded with the gratings in different orientations. In general, SIM enhances the resolution only by a factor of 2 (because the SIM pattern cannot be focused to anything smaller than half the wavelength of the excitation light). However, further increases in resolution can be achieved by introducing nonlinearities, which show up as higher-order harmonics in the image. SIM is very useful in the modern imaging lab because, despite the relatively modest enhancement of resolution it is compatible with most any fluorophore. An example of multicolor SIM recorded as a part of studies into cell migration during cancer54 is shown in the figure illustration. In this particular case, the cytoskeleton of a migrating human glioma cell has been targeted by immunocytochemistry to reveal three abundant cytoskeletal protein networks inside a single cell: microtubules, vimentin, and actin microfilaments.

The common theme among the aforementioned Nobel Prize laureates was that their specific contributions considerably enhanced the ability of biological imaging to be applied across mesoscopic scales, ie, helped build the connection between the microscopic and macroscopic worlds, thereby adding another critical scale of dimension required to understand living biology.

Understanding the biology of life requires imaging across scales from macroscopic to microscopic

It is spatial and temporal resolution of imaging technologies that allow us to see and appreciate biological function across the scales. Hand in hand with the understanding derived from such advances in spatial resolution as obtained with SR and electron microscopy, the use of GFP has fueled the emergence of an unprecedented capacity to image at the macroscopic level.63,64 Arguably, one of the most important advances in modern imaging microscopy for research applications has been the recent development of techniques allowing long-term visualization of complete cell level dynamics in the context of relatively large living tissue samples like, for example,
embryogenesis, and cardiac muscle contraction using so-called light-sheet technologies. This has resulted in the need for a system-level understanding of biology, ultimately opening the way to a full description of living organisms and disease that takes into account the molecular, cellular, tissue, organ, and organism scales. Systems biology asserts that beyond a complete description of the physiology in which biology arises from molecular physicochemical processes, it is essential to complete an integrative approach to the biology of life and the treatment of pathology and disease. In this context, the need to appreciate the microscopic to macroscopic scales for visualization of living biology is essential. In our modern, postgenomic world, to help guide diagnosis, a medical doctor, pathologist, or other health worker will use a battery of biochemical tests to better identify endocrine, immune, genetic, infectious, and microbial imbalance. Where necessary, imaging will be used, such as x-ray CT, MRI, PET, and ultrasound, allowing noninvasive visualizing of bodily structure and function. These biomedical technologies have revolutionized clinical care by providing images with millimeter precision of a bone fracture, dental decay, a collapsed lung, a tumor mass, an obstructed intestine, neonatal development, or kidney stones, among many other possibilities. Ever more advanced imaging techniques, often combined with sophisticated contrast probe chemistries, can reveal the accumulation of a specific marker in potentially cancerous tissues, fluid accumulation, inflammation, and even bacterial or viral infection.

Ultrastructural characterization of Shigella invasion site using large-volume correlative light electron microscopy (CLEM). Fluorescence confocal microscopy was used to identify and characterize the invasion site of Shigella bacteria into an epithelial cell; this was immediately followed by large volume focussed-ion-beam scanning electron microscopy (FIB/SEM) of the same invasion site and bacteria. Top right panel: conventional fluorescence image revealing the target cell nucleus (blue) and an endogenous signaling protein under study in this work (green; Rab11-eGFP) (scale bar, 5 μm). Top left panel: shows a zoomed subcellular region of interest where invading bacteria are detected plus Rab11-eGFP vesicles (scale bar, 1 μm). Lower panels: FIB/SEM image of the same invasion site (lower left corner, scale bar 1 μm). Lower center and right panels: using an imaging software, the bacteria-containing vacuoles and host vesicles were segmented from the 3-D EM data set (shown in blue and orange, respectively). Fluorescent Rab11-positive vesicles (green) were correlated with the host vesicles (orange). Images adapted from reference 62. Experiments designed and performed by Nora Mellouk, Allon Weiner & Jost Enninga, Unité de Dynamique des Interactions Hôte-Pathogène, Institut Pasteur).

Clinical diagnosis and therapies require not only data at a macroscopic level, but also data to qualify and quantify what is happening at the microscopic cellular level.\textsuperscript{86-92} For example, a pathologist can assess the grade of a tumor based on microscopic analysis of cellular morphology from a biopsy sample. However, biopsy is by definition invasive, and can in itself augment cost and risk. This risk arises because noninvasive clinical imaging techniques are currently mostly restricted to macroscopic analysis, and conventional biopsy remains the only recourse for microscopic analysis critical to high-quality precision care.

The dichotomy between the macroscopic and microscopic world has its basis in physics, as penetration of biological tissues is dependent on the wavelengths of the electromagnetic energy spectrum.\textsuperscript{74,75,78,93} Consequently, the spatial resolution limits of molecular imaging modalities are in the range of millimeters to centimeters, far from the micron range required for microscopic analysis of cellular level organization. Electromagnetic wavelength ranges suitable to detect with appropriate resolution cellular structure and beyond in optical and electron microscopy are perfect for high-contrast detection of microscopic structure. However, in this case the laws of physics severely limit the passage of these wavelengths inside living tissues,\textsuperscript{64} making it necessary to use optical modalities,\textsuperscript{86,87,92,94,95} in particular those requiring not only new probe chemistries capable of recognizing disease tissue, but also new hardware and optical imaging modalities that can be combined with clinical intervention such as surgery. For example, during the last 10 years, a Paris-based startup, Mauna Kea Technologies (MKT), has developed innovative fiberoptic confocal videomicroscopy devices able to provide a near-field microscopic view of cellular tissue during surgical intervention,\textsuperscript{96-100} which enables morphological analysis through “optical biopsy” of tissues in situ. Anecdotally, MKT founder, Sacha Loiseau started his career in astrophysics, working on astronomy projects at NASA, such as technologies to generate clear images of the faintest objects millions of miles from earth. Having seen the imaging of the universe’s farthest frontiers become a reality, he then dedicated himself to finding a way to clearly visualize the inside of the human body using astrophysics instrumentation applied to medical imaging.

**Epilogue—“mesoscopy,” the real scale of things**

It is remarkable that only 150 years ago the microscopic world was barely conceptualized. Indeed, the discovery of the microscopic, nanoscopic, and molecular/atomic scales during the last 350 years still resonates as the major driving force powering the innovation of today’s clinical and biomedical research technologies. Invention of the visible light–based microscope opened up a whole new world of microbiological, cellular, and biochemical understanding. In this context, the reader will no doubt enjoy visiting the Molecular Expressions web resources at: http://micro.magnet.fsu.edu/primer/java/scienceopticsu/powersof10/. This wonderful animation is premised by the observation that the earth and a single plant cell are 12 orders of magnitude apart in physical dimension: Earth = 12.76×10\(^{+6}\) = 12.76 million meters in diameter Plant cell = 12.76×10\(^{-6}\) = 12.76 millionths of a meter across

It is a further sobering thought that subatomic particles are some 12 orders of magnitude smaller than the nucleus of the plant cell. This is about the same order of magnitude that dwarfs the Earth’s diameter compared with that of our galaxy, the Milky Way. It follows that the journey ahead to understand the molecular biology of a single cell at a systems-level of understanding may be compared, in astrophysics, with the size of the Earth with respect to the known universe. Similar to space exploration, deeper understanding of life sciences and biology shall continue to depend upon innovative imaging technologies able to aid our visualization at these mesoscopic scales.
Innovative biological technologies: cracking the cell’s ultimate secrets? –

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