The challenging landscape of hematological malignancies: recent advances

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Immunotherapy and the future management of hematological malignancies – Mohty

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Historically, there have been limited options for the treatment of hematological malignancies (HMs), particularly in patients with high-risk disease features, poor prognosis, or relapsed or refractory disease. However, today, the treatment paradigm of several HMs is radically shifting towards new concepts, especially with the introduction of different forms of immunotherapy approaches and modalities.

The first form of immunotherapy to be used in HM was allogeneic hematopoietic stem cell transplantation (allo-HSCT), which has a curative potential for many aggressive HMs. However, the use of allo-HSCT and its success are limited by an unpredictable immune reaction, the so-called graft-versus-host disease (GVHD) phenomenon. A clinically significant GVHD and its corollary of morbid sequelae and mortality can affect more than 50% of patients undergoing allo-HSCT. On the other hand, clinically moderate GVHD is usually related to a simultaneous immune-mediated graft-versus-tumor (GVT) effect, which can allow for long-term disease control and cure. Understanding and controlling the separation between GVHD and the GVT effect is usually considered the holy grail in the field of hematology. Today, with the advent of novel and innovative immunotherapeutic approaches, the role of allo-HSCT, in combination or not with the many new effective drugs becoming available, is being challenged and needs to be refined.

While allo-HSCT is the most widely used form of immunotherapy in HM, the history of cancer immunotherapy dates back to more than a century ago. Coley first described so-called “Coley toxins,” which were supposed to induce regression of established inoperable tumors. Later on, the use of Bacillus Calmette-Guerin (BCG) for the treatment of some forms of bladder cancer offered another illustration of the value of immunotherapy in cancer, which brought in the tantalizing possibility of inducing long-term, durable remissions and cure when harnessing the immune system of the patient. The next major milestone in immunotherapy was the discovery in the late 1970s and early 1980s of cytokines such as interferon alpha (IFN-α) and interleukin 2 (IL-2). Indeed, besides their key role in antiviral immunity, accumulating evidence indicated that these immunostimulatory or immunomodulatory cytokines could also control the major pathways underlying cancer immunosurveillance. Against this background, numerous clinical studies were performed and demonstrated that these cytokines could mediate regression of some established malignant tumors. This finding led to the approval of IFN-α and recombinant IL-2 for the treatment of malignancies, especially metastatic renal cell carcinoma and metastatic melanoma; some patients were disease-free more than 20 years after therapy. However, the use

Address for correspondence:
Mohamad MOHTY, MD, PhD
Hematology and Cellular Therapy Department,
Hôpital Saint Antoine
Paris, FRANCE

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of cytokines in cancer immunotherapy did not translate into an overall survival advantage and was limited by many challenges, including unacceptable toxicity.

In parallel with the use of immunomodulatory cytokines, researchers were actively working on the identification of tumor-associated antigens (TAAs), which were thought to be a mandatory step towards developing effective adoptive cellular immunotherapy. While dozens of TAAs have been identified and successfully characterized, adoptive cellular immunotherapy has proven to be more complex than initially thought and remains a marginal option in the treatment of cancer and HM. Moreover, adoptive T-cell therapy has been mainly applied to those melanoma patients for whom an adequate culture and expansion of isolated tumor-infiltrating lymphocytes (TILs) can be elicited. The main barriers to this approach have been the difficulty in standardizing the culture and manufacturing of TILs, the capacity of tumors to induce a potent immune tolerance status, and the requirement for major histocompatibility complex (MHC) presentation of antigens.

Despite the above limitations, the characterization of TAAs and TILs spurred the development of an impressive variety of therapeutic cancer vaccines, using peptides, whole proteins, allogeneic and autologous dendritic cells, recombinant viruses, whole cells, and plasmid DNA. However, one must acknowledge that most (if not all) of these vaccine strategies have failed, probably as a consequence of the immunosuppressive tumor microenvironment that first has to be overcome in order to make these approaches more widely applicable and effective. In fact, overcoming central tolerance is likely to be the most challenging barrier to efficient immunotherapy. One approach to achieving this is represented by the transfer of alloreactive effector cells, as done in the context of allo-HSCT with all of its limitations described above.

Increased knowledge about the relevance of peripheral tolerance in blunting the antitumor immune response led to the development and evaluation of antibodies or molecules that act on crucial immune checkpoint inhibitors, which are used by malignant cells to evade the immune system. From this perspective, blocking the activity of the protein receptors CTLA-4 (cytotoxic T-lymphocyte antigen 4 or CD152) and PD-1 (programmed death 1 or CD279) has resulted in potent antitumor activity. These results have led to the first-in-class approval of an anti-CTLA-4 antibody in solid tumors and the approval of PD-1–modulating agents in HM and different solid tumors. The rapid advent of immune checkpoint inhibitors has opened a new avenue for the treatment of aggressive HM, which has been especially characterized by the success of tumor-specific monoclonal antibodies (mAbs) since the year 2000. Of particular note, the mAb directed against the CD20 antigen in lymphoid HM is a remarkable success story, being both safe and effective over the long term. The development of combined treatment packages, including mAbs, immune checkpoint inhibitors, highly selective agents for direct therapeutic modulation of regulated apoptotic pathways, and cellular therapies, is becoming less hype and more reality in many yet-incurable HMs.

The engineering of gene-modified T cells directed at well-established tumor-specific target antigens is proving to be very attractive, because it offers the possibility of exploiting both the antigen-binding property of mAbs and the lytic and self-renewal capacities of T cells. This is the concept behind chimeric antigen receptor–expressing (CAR) T cells, which are the best example of modern and innovative adoptive cellular immunotherapy to emerge from more than 50 years of research efforts. CAR T cells can recognize and kill tumor cells independently of MHC, and they have the added benefit of a rapid onset of action (as in chemotherapy), while circumventing both immune tolerance of the T-cell repertoire and major histocompatibility restriction. The first CAR T cells to be tested have been directed at HMs, given the extent of surface antigens known to be expressed on HM cells, the relative ease of tumor sampling, and the natural preference of T-cell homing towards hematologic disease sites, such as the peripheral blood, bone marrow, and lymph nodes. Thus far, most clinical trials of CAR T cells in HMs have focused on B-cell malignancies, targeting CD19 or CD20. The CAR design, manufacturing process, and results have varied from one trial to another, but a common toxicity profile appears to have emerged; namely, in most cases, cytokine release syndrome, which seems to correlate with antitumor activity.

The advent and introduction into the clinic of CAR T cells is occurring simultaneously with the development of various engineered monospecific antibodies and bispecific antibodies, such as the dual affinity re-targeting (DART) antibodies, and another class of bispecific antibody constructs, called bispecific T-cell engagers. The latter have been designed to di-
rect effector memory T cells towards target tumor cells. The proximity induced by bispecific T-cell engagers triggers target cell–specific cytotoxicity, which closely resembles conventional cytotoxic T-lymphocyte activation. This T cell–mediated target-specific killing is the therapeutic mechanism of recently approved agents such as blinatumomab, which specifically targets cells that express CD19. Then, blinatumomab recruits and activates T cells via interaction with CD3+ T cells. These activated T cells induce target cell lysis, demonstrating that blinatumomab is an extremely potent molecule.

All of the above developments are introducing a true revolution in the field of hematology, and they represent a big step forward towards new, approved therapies, which will shift the treatment paradigm of most HMs. In the near future, these innovative approaches will complement conventional multi-agent chemotherapy, but may also rapidly pave the way for chemotherapy-free treatment in some specific situations. Treatments in the field of hematology are now making the transition from being “merely promising” to “highly effective,” even in the most aggressive blood diseases. Also, it is reasonable to anticipate their broader and more rapid application beyond relapsed and refractory situations. Frontline therapeutic protocols incorporating these new approaches are being tested, but deciding on what is the optimal sequence of each therapy in different protocols and on how to alternate drugs to prevent tumor resistance to therapy are key questions that now need to be tackled. All these changes will enhance the field of hematology. There should be no room for timidity in our approach to change, as change will provide fresh hope and opportunity for those patients who are most in need. The pace of change is accelerating. Hematology as a discipline is committed to staying at the forefront, or ahead, of the game.

**Keywords**: allogeneic hematopoietic stem cell transplantation; immune checkpoint inhibitor; chimeric antigen receptor; dual affinity re-targeting antibody; monoclonal antibody; tumor-associated antigen
Apoptosis is a process of programmed cell death central to tissue development and homeostasis in the mature organism. The BCL-2 family of proteins regulates apoptosis in response to cellular stress. Downregulation of apoptosis is a hallmark of cancer, conferring resistance to stress signals and to conventional anticancer therapy. Because of the nature of the interface between members of the BCL-2 family, drug development of "BH3 mimetics" has been challenging. Yet, there have been notable successes in the development of small molecules that can reinstate apoptosis in cancer cells, with three drugs currently progressing through clinical trials. ABT-199/venetoclax, a selective and orally bioavailable small molecule inhibitor of BCL-2, has recently been approved by the Food and Drug Administration for the treatment of patients with chronic lymphocytic leukemia with 17p deletion who have received at least one prior therapy. In parallel, continued efforts have been devoted to targeting other prosurvival proteins to expand the range of tumors that could be efficiently treated with BH3 mimetics. Altogether, the preclinical and clinical data allow us to envision a promising future for BH3 mimetics.

While small molecules with different selectivity profiles for prosurvival proteins have not yet reached the clinic, ABT-263 and its successor ABT-199 have demonstrated the feasibility of the approach, the relative safety of inducing apoptosis in cancer patients, and, more importantly, the efficacy of such treatments.

Address for correspondence:
Seong Lin Khaw, ACRF Chemical Biology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria; Department of Medical Biology, The University of Melbourne, Victoria; The Royal Children’s Hospital, Parkville, Victoria; Department of Pharmacology and Therapeutics, The University of Melbourne, Victoria, AUSTRALIA

Guillaume Lessene, PhD
1ACRF Chemical Biology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria; Department of Medical Biology, The University of Melbourne, Victoria; 3The Royal Children’s Hospital, Parkville, Victoria; Department of Pharmacology and Therapeutics, The University of Melbourne, Victoria, AUSTRALIA

A promising future for inducers of apoptosis

by S. L. Khaw and G. Lessene, Australia

...most currently used conventional and targeted anticancer therapies require functional apoptotic signaling to exert their activity. For example, proapoptotic BIM protein has been found to be crucial for the activity of corticosteroids in lymphoid malignancies, of tyrosine kinase inhibitors (such as imatinib and gefitinib), and of mitogen-activated protein kinase (MEK) inhibitors. Similarly, induction of PUMA by p53 mediates the activity of genotoxic chemotherapeutic drugs...
BCL-2 (B-cell lymphoma 2) family of proteins converging on the mitochondria. Both pathways eventually induce the activation of cysteine proteases, caspases, which provoke the well-known morphological features of apoptosis: cellular component dismantlement, DNA-laddering, and cellular blebbing. In this review, we will primarily focus on the BCL-2–driven intrinsic pathway and how the understanding of its biology has led to a new paradigm in cancer treatment.

BCL-2–driven apoptosis in normal and cancer cells.

Regulation of the upstream apoptotic cascade by the BCL-2 family of proteins in normal cells (A). In cancer cells (B), overexpression of prosurvival proteins blocks normal apoptosis and leads to cancer cell survival. Small molecule BH3 mimetics antagonize the prosurvival activity of the BCL-2 proteins (C) and reintroduce apoptosis in cancer cells.

Abbreviations: BAD, BCL-2 antagonist of cell death; BAK, BCL-2 antagonist/killer; BAX, BCL-2-associated X protein; BCL-2; B-cell lymphoma 2; BCL-X1, B-cell lymphoma-extra large; BFL1, BCL-2-related protein A1; BH3, BCL-2 homology domain 3; BIM, BCL-2 interacting mediator of cell death; MCL1, myeloid cell leukemia 1 [protein]; PUMA, p53 upregulated modulator of apoptosis.

The critical regulators of the intrinsic, or mitochondrial, pathway are the BCL-2 family of proteins. Members of this family fall into one of two main protein classes, depending on their ability to induce or repress cell death. The subclass that comprises BCL-2 itself, BCL-X1 (B-cell lymphoma-extra large), BCL-W, MCL1 (myeloid cell leukemia 1), and BFL1 (BCL-2-related protein A1 [in mouse]) has prosurvival activity and therefore maintains cellular viability (Figure 1). In a healthy cell, their action opposes the prodeath activity of two classes of proapoptotic proteins. The first class of pro-apoptotic proteins includes BAX (BCL-2 associated X protein) and BAK (BCL-2 antagonist/killer), which reside primarily at the mitochondria and function as the “executioners” of apoptosis, as their activation is believed to be a point-of-no-return in the apoptotic process.2 By forming oligomers at the mitochondrial surface, BAX and BAK create pores that allow the downstream apoptotic cascade to proceed through the release of cytochrome c and activation of caspases. Another class of proapoptotic proteins acts as initiators of the apoptotic cascade responding to stress signals. These are structurally more distant proteins and are called “BH3 (BCL-2 homology domain 3)-only proteins” as they only retain one domain common to the whole BCL-2 family (the BH3 domain). Upon receipt of stress signals (eg, loss of attachment, cytokine withdrawal, DNA damage, or infection), these BH3-only proteins are unleashed and antagonize the prosurvival proteins or directly activate BAX and BAK (Figure 1A).

Deregulation of cellular lifespan by alteration of the apoptotic machinery is a hallmark of a large number of life-threatening diseases ranging from cancer (too little apoptosis [Figure 1B]) to ischemia reperfusion events (too much apoptosis).2 We will focus on the former, for which downregulation of apoptosis is a major contributing factor. As nicely captured by Hanahan and Weinberg,3,4 it is now understood that cancer arises from a combination of critical changes at the gene level impacting cellular physiology. While the capacity for self-replication and uncontrolled growth have long been recognized...
as key characteristics of neoplasms, it was later found that these events must be accompanied by abrogation of the apoptotic response, as apoptosis in fact represents a key intrinsic defense against oncogenic mutations.

Neoplastic transformation is associated with multiple mechanisms of apoptotic downregulation. These include mutations in key tumor suppressors such as p53 (which normally functions at least in part by coupling cellular stresses associated with cancer, such as DNA damage, oncogene activation, and hypoxia, to the activation of apoptosis) as well as overexpression of prosurvival factors, such as BCL-2, BCL-X, or MCL1, or suppression of proapoptotic proteins, such as BIM (BCL-2 interacting mediator of cell death) (Figure 1C). The contribution of impaired apoptosis to cancer initiation and maintenance has now been amply demonstrated both in hematological and solid tumors. Since the details for the biology underpinning the role of the BCL-2 family of proteins (Figure 1D) has been detailed in a number of recent reviews, we will present here only key aspects and focus on the recent development of small molecules and their impact on hematological malignancies. This review will also limit itself to data associated with “validated” BH3 mimetics, ie, compounds for which there is convincing evidence that their cytotoxicity is entirely attributable to direct targeting of prosurvival BCL-2 family proteins.

The BCL-2 family in the hematological compartment

Blood cells are constantly generated and discarded either as part of a general homeostatic process (normal replacement of blood cells) or in response to stresses such as inflammation or infection. Apoptosis, especially through the BCL-2 family of proteins, acts at multiple steps of this process to provide a high level of control required not only for the regulation of the number of blood cells, but also for the critical selection of desired cellular phenotypes (summarized in reference 7). Three examples illustrate the role of BCL-2 proteins. Firstly, apoptosis is critical during the maturation of thymocytes and deletion of autoreactive T cells. Deficiency in the proapoptotic sensor, BH3-only protein BIM allows autoreactive T cells to persist instead of being forced to undergo apoptosis. This defect results in autoimmune diseases. Prosurvival proteins such as BCL-2 and A1 have also been implicated in T-cell maturation. Secondly, in the B-cell compartment, the prosurvival protein MCL1 plays a role in the survival of long-lived plasma cells, while BCL-2 is involved during earlier stages of B-cell maturation. Finally, a most striking role for apoptosis was observed in determining the lifespan of platelets, small anuclear cells shed from progenitor megakaryocytes. It was shown that platelet survival is underpinned by the balance between the prosurvival protein BCL-XL and proapoptotic protein BAK. Since BCL-XL half-life is shorter than that of BAK, platelets eventually die by apoptosis when BAK’s proapoptotic activity is left unrestrained.

Altogether the role of BCL-2 proteins in normal blood physiology is an important factor to take into account when thinking about targeting these proteins with small molecules. While pharmacological modulation of the BCL-2 family of proteins may not always phenocopy genetic deletion, a wide range of “on-target” hematological side effects could accompany treatment with small molecules targeting the apoptotic pathway.

The BCL-2 family and hematological cancers

The BCL-2 family has been shown to be a critical factor not only for the initiation of hematological malignancies, but also in their maintenance. BCL-XL and MCL1 copy number were found to be amongst the most elevated genes in a study looking at a large number of cancers, including hematological malignancies. The prosurvival protein BCL-2 itself has been found to be a key driver of multiple lymphoid tumors. Its oncogenic role was initially discovered through the study of follicular lymphoma, where the characteristic t(14;18) translocation deregulates BCL-2 gene expression, leading to elevated protein levels. Similarly, in chronic lymphocytic leukemia (CLL), the almost universal overexpression of BCL-2 is due to loss of critical regulatory microRNAs (miR-15a and miR-16-1) underpins both leukemogenesis and disease progression. MCL1 and, to a lesser extent, BCL-2 were shown to have important roles in sustaining multiple myeloma cell survival.

Conversely, the downregulation or complete silencing of expression of proapoptotic BH3-only proteins, which act as tumor suppressors, has also been demonstrated in several cancers. For example, BIM levels are decreased in B-cell lymphomas through allele deletion or in infant acute lymphoblastic leukemia via epigenetic suppression. Expression of PUMA (p53 upregulated modulator of apoptosis), a key transcriptional target of the tumor suppressor p53, has been found to be very low in 40% of Burkitt lymphoma.

Data associated with these human genetic studies have been supported by numerous experiments using genetically engineered mice. In a seminal experiment by Strasser and colleagues, concurrent overexpression of Bcl-2 was demonstrated to greatly accelerate the development of E-myc lymphoma. Notably, isolated overexpression of Bcl-2 in the lymphoid compartment was itself associated with spontaneous lymphomagenesis, albeit with much lower penetrance and greater latency than overexpression of classical oncogenes such as myc. This implicated, for the first time, the capacity of perturbed cell death to not only directly facilitate neoplastic transformation, but also to potentiate the effect of other oncogenes. This illustrates the role of impaired cell death in permitting cancer cell survival in spite of activated cellular stress pathways (triggered, for example, by uncontrolled cell growth), which normally converge on activation of the intrinsic apoptotic pathway. The dependency of myc-driven tumors on prosurvival proteins has subsequently been observed in other models. Mouse E-myc and its human counterpart
Burkitt lymphomas are dependent on MCL1.28 Mouse models of acute myeloid leukemia (AML) have also highlighted the role of MCL1 in this disease.29

It is also important to note that most currently used conventional and targeted anticancer therapies require functional apoptotic signaling to exert their activity. For example, the proapoptotic protein BIM has been found to be crucial for the activity of corticosteroids in lymphoid malignancies,34 of tyrosine kinase inhibitors (such as imatinib and gefitinib), and of mitogen-activated protein kinase kinase (MEK) inhibitors.30,31 Similarly, induction of PUMA by p53 mediates the activity of genotoxic chemotherapeutic drugs (such as alkylating agents, topoisomerase inhibitors, and anthracyclines).25 Not surprisingly, impaired apoptosis has now been recognized as a key mechanism of chemoresistance. For example, MCL1 stabilization or BCL-XL overexpression have been associated with resistance to a variety of cytotoxic therapies.32,33

This spawned the therapeutic hypothesis that reactivation of apoptosis in cancer cells could prove efficacious in treating cancers resistant to currently available treatments. From the outset, a number of questions arose regarding the feasibility of such an approach. Firstly, would this promising rationale translate into a clinical benefit in patients? Secondly, since the idea underpinning this strategy was to trigger the apoptotic pathway, which is present in all cells, would a useful therapeutic window exist, ie, would it target cancer cells with sufficient selectivity over normal cells? Finally, would it be possible to even develop compounds targeting this new class of proteins?

**Development of BH3 mimetics**

Upstream of the mitochondria, the apoptotic cascade is orchestrated by interactions between members of the BCL-2 family of proteins (Figure 1). These interfaces are typically represented as “helix-in-groove” between prosurvival and pro-apoptotic proteins (Figure 2).34 The surfaces to target with a small molecule are very large and in the case of the BCL-2 family, shallow and mainly hydrophobic.36 These structural features represented a challenge to medicinal chemists, whose aim was to develop compounds that would replicate the functional activity of the BH3-only proteins, thus inducing apoptosis, by interacting directly with the binding groove of the prosurvival proteins. The difficulty of the task is illustrated by the fact that only a very small number of validated BH3 mimetics have reached the clinic; this after more than 20 years of biological discoveries validating the importance of the pathway in cancer. Nevertheless, success stories have been reported with these few compounds now in the clinic.

- **The early years: ABT-737 and ABT-263**

  AbbVie started a program targeting the BCL-2 family of proteins using an emerging strategy, structure-activity relationship by nuclear magnetic resonance ("SAR by NMR"),36 a technique using fragments as a starting point for drug discovery. This endeavor culminated in the development of ABT-737,36,37 While a number of small molecules had been reported to target the pathway, ABT-737 was the first to achieve the properties of a bona fide BH3 mimic. It demonstrated very high affinity for three prosurvival proteins (BCL-2, BCL-XL, and BCL-W),37 which translated into very potent cellular activity in cancer cells and crucially was confirmed to act by direct inhibition of the prosurvival activity of principally BCL-2 and BCL-XL.38,39 ABT-737 has since proven an invaluable tool not only for studying apoptosis, but also for understanding the efficacy as well as possible toxicities of BH3 mimetics.37 In pre-clinical models, ABT-737 has demonstrated potent single-agent activity in hematological tumors40 and in a subset of non-small cell lung carcinomas,41 as well as in combination with targeted cytotoxics (such as MEK inhibitors)42 or conventional cytotoxics (such as docetaxel)42 in a variety of other malignancies. ABT-737 also confirmed the role of BCL-XL in maintaining platelet viability.13

  Despite the enormous amount of work required to develop ABT-737, this molecule suffered from a poor pharmacological profile precluding its progression to clinical trial. Additionally, drug optimization ensued and delivered ABT-263, a compound exhibiting the same target profile as ABT-737, but with oral availability and properties suitable for progression to clinical trial.43 Preclinical data with ABT-263 essentially mirrored the efficacy of ABT-737 in hematological malignancies,44 both as a single agent and in combination with other drugs.45,46 Phase 1/2a clinical trials with ABT-263/navitoclax have now been completed in a variety of lymphoid malignancies and sol-
Despite the early phase setting (where trials are designed to establish safety rather than efficacy), striking clinical responses were observed, particularly in patients with CLL. Notably, significant responses were observed even in heavily pretreated patients with CLL bearing 11q deletion or 17p deletion, genomic aberrations that predict very poor prognosis following contemporary immunochemotherapy. Amongst the patients treated with clinically efficacious doses, 35% exhibited partial responses, though no complete responses were observed. ABT-263/navitoclax was also well tolerated, with observed toxicities—such as fatigue and diarrhea—that were generally manageable. As anticipated from preclinical studies, dose-limiting toxicity was from acute-but-reversible thrombocytopenia. Modified dosing schedules were successfully implemented to ameliorate this on-target toxicity. ABT-263/navitoclax is a landmark molecule in this field. It was the first validated inhibitor of prosurvival proteins to demonstrate clinical efficacy, confirming the feasibility of reinstating apoptosis in the cancer cell to treat cancer.

The development of ABT-263 was hampered by thrombocytopenia, which significantly limited achievable clinical exposures. Importantly, it was subsequently recognized that most of its efficacy in CLL arose from its targeting of BCL-2. Developing a compound to selectively target BCL-2 therefore became a priority: such a compound would have the advantage of superior safety, as well as potentially greater efficacy through higher achievable dosing. Currently, two BCL-2–selective BH3 mimetics have reached the clinic: one developed

### Table I. Clinical trials of BH3 mimetics.

<table>
<thead>
<tr>
<th>Molecule (origin)</th>
<th>Single agent/combination</th>
<th>Phase</th>
<th>Indication(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABT-263/navitoclax (AbbVie/Genentech)</td>
<td>Single agent</td>
<td>1/2</td>
<td>CLL, refractory CLL, FL, CTCL, NHL, indolent lymphoma, MCL, PTCL</td>
</tr>
<tr>
<td></td>
<td>Single agent</td>
<td>1</td>
<td>NHL, CLL, MCL, PTCL, TCL, FL, lymphoma</td>
</tr>
<tr>
<td></td>
<td>Single agent</td>
<td>1</td>
<td>Relapsed CLL</td>
</tr>
<tr>
<td></td>
<td>Combination with gossypol</td>
<td>2</td>
<td>CLL</td>
</tr>
<tr>
<td></td>
<td>Combination with rituximab</td>
<td>2</td>
<td>CLL</td>
</tr>
<tr>
<td></td>
<td>Combination with vinorelbine</td>
<td>1</td>
<td>FL, CLL, relapsed CLL, refractory CLL</td>
</tr>
<tr>
<td></td>
<td>Combination with rituximab</td>
<td>1</td>
<td>CLL, HL, DLBCL, LL</td>
</tr>
<tr>
<td></td>
<td>Combination with paclitaxel</td>
<td>1</td>
<td>Hematological tumors, lymphoma</td>
</tr>
<tr>
<td></td>
<td>Combination with etoposide/cisplatin</td>
<td>1</td>
<td>Hematological tumors, lymphoma</td>
</tr>
<tr>
<td></td>
<td>Combination with fludarabine/ cyclophosphamide/rituximab or bendamustine/rituximab</td>
<td>1</td>
<td>Refractory CLL, CLL, lymphoma</td>
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<tr>
<td>ABT-199/venetoclax (AbbVie/Genentech/ WEHI)</td>
<td>Single agent</td>
<td>3</td>
<td>CLL, relapsed CLL, refractory CLL</td>
</tr>
<tr>
<td></td>
<td>Single agent</td>
<td>2</td>
<td>Relapsed CLL, refractory CLL, CLL, MM, AML, refractory AML, relapsed AML</td>
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<tr>
<td></td>
<td>Single agent</td>
<td>1</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>Single agent</td>
<td>1</td>
<td>CLL, MCL, AML, MM, NHL, refractory MM, relapsed MM, AML, leukemias</td>
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<tr>
<td></td>
<td>Combination with ibritinib</td>
<td>2</td>
<td>Refractory CLL, relapsed CLL, CLL, MCL</td>
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<tr>
<td></td>
<td>Combination with obinutuzumab</td>
<td>2</td>
<td>CLL, DLBCL</td>
</tr>
<tr>
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<td>Combination with bendamustine + rituximab compared to ABT-199 + rituximab</td>
<td>1/2</td>
<td>AML, refractory AML, relapsed AML, FL, relapsed CLL</td>
</tr>
<tr>
<td></td>
<td>Combination with cobimetinib and combination with idelalisib</td>
<td>1/2</td>
<td>Refractory CLL, lymphoma, NHL, CLL, DLBCL, indolent lymphoma, MCL, TCL</td>
</tr>
<tr>
<td></td>
<td>Combination with duvelisib</td>
<td>1/2</td>
<td>Refractory CLL, lymphoma, NHL, CLL, DLBCL, indolent lymphoma, MCL, TCL</td>
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<tr>
<td></td>
<td>Combination with polatuzumab, vedotin, and obinutuzumab</td>
<td>1/2</td>
<td>FL, NHL, DLBCL, BCL</td>
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<td></td>
<td>Combination with obinutuzumab and ibritinib</td>
<td>1/2</td>
<td>MCL, NHL, DLBCL, B-cell NHL</td>
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<tr>
<td></td>
<td>Combination with obinutuzumab and ibrutinib</td>
<td>1/2</td>
<td>Relapsed CLL, refractory CLL, CLL</td>
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<tr>
<td>SS5646 (Servier/Novartis)</td>
<td>Combination with idelalisib</td>
<td>1</td>
<td>FL, MCL</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myelocytic leukemia; BH3, Bcl-2 homology domain 3; CLL, chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HL, Hodgkin lymphoma; LL, lymphoblastic lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; PTCL, peripheral T-cell lymphoma; TCL, T-cell lymphoma; WEHI, Walter and Eliza Hall Institute [of Medical Research].

Data from Pharma eTrack (www.pharmaetrack.com), Abbvie.com, and clinicaltrials.gov.
by Servier (S55746) and progressed through clinical trials together with Novartis; and the other (ABT-199/venetoclax) originating from AbbVie, developed in collaboration with Genentech and the Walter and Eliza Hall Institute. Based on the ABT-737/-263 scaffold, ABT-199 was designed to take advantage of key structural features in the binding pocket of BCL-2 to achieve not only higher selectivity, but also higher affinity for this target.66

Encouragingly, preclinical studies confirmed that ABT-199 as a single agent recapitulated the activity of ABT-737 and ABT-263 in CLL and B-lymphoid malignancies.55,56 ABT-199 also demonstrated in vitro activity in myeloid/lymphoid or mixed-lineage leukemia–rearranged AML,19 as well as multiple myeloma.55,56 In addition, efficacy was observed in animal models of myc-driven lymphomas overexpressing Bcl-2.60 Notably, human high-grade lymphomas characterized by concurrent BCL-2 overexpression and myc rearrangements (termed double-hit lymphomas) have markedly inferior outcomes following treatment with contemporary frontline immunotherapeutic regimens.51

Data from the ABT-199 phase 1 clinical trial in patients with CLL or small lymphocytic leukemia have recently been published,59 presenting compelling validation of the rationale that underpinned its development. Removal of affinity for BCL-XL indeed abrogated the thrombocytopenia seen with ABT-26355 and, while other adverse events were observed, they were manageable. Dosing was escalated to 1200 mg/day (compared to a maximum tolerated dose of 250 mg/day with ABT-263/venetoclax) without dose-limiting toxicity, and no maximum tolerated dose was defined. Notably, the most significant adverse event that impacted the early stages of this trial was tumor lysis syndrome (TLS), due to the rapid destruction of CLL tumor cells. While this potentially life-threatening phenomenon was concerning, it also indicated the drug’s antileukemic potency. Changes in protocol with the introduction of a low-dose challenge one week prior to commencing daily ABT-199, followed by gradual dose escalation to the target cohort dose for all patients averted further episodes of clinical TLS. Across all dose cohorts, the overall response rate was 79%, with 20% of patients achieving a complete response, including 5% of the overall cohort where flow cytometry-based minimal residual testing in bone marrow was negative. Strikingly, responses were observed in all dose cohorts and across all patient subgroups. In particular, response rates were independent of prior responsiveness to fludarabine, IgV_{\mu} mutation status, or the presence of adverse genomic aberrations (including 11q deletion and 17p deletion). These are remarkable results for a single-agent phase 1 clinical trial.

ABT-199/venetoclax has since progressed to phase 3 clinical trials in relapsed and refractory CLL in combination with monoclonal anti-CD20 antibody therapy (Table I). In addition, more than 20 phase 1 or 2 clinical trials involving ABT-199 as monotherapy or in combination with standard or emerging therapies (such as ibrutinib) are in progress across a range of hematological malignancies. In April 2016, the US Food and Drug Administration (FDA) accelerated approval of ABT-199/venetoclax in the treatment of patients with chronic lymphocytic leukemia with 17p deletion who have received at least one prior therapy. As mentioned above, Servier has also launched a clinical trial with its own BCL-2–selective agent S55746 in patients with follicular lymphoma and mantle cell lymphoma (MCL). It still remains to be seen how this molecule compares with ABT-199.

**ABT-199 in other malignancies**

ABT-199 has also shown promising activity preclinically against AML.52,54 Of all the prosurvival proteins not targeted by ABT-199, MCL1 was found to be an important factor for resistance.65,66 As a result, multiple strategies have been deployed to indirectly impact MCL1 levels. These attempts include a combination with an inhibitor of NEDD8 (neural precursor cell expressed, developmentally downregulated 8) in AML,55 suggesting that inhibition of MCL1 by NOXA combined with ABT-199 has a strong effect in this context. Similarly, combination with dinaciclib in diffuse large B-cell lymphoma (DLBCL) decreased levels of MCL1 and potentiated ABT-199’s activity.68 Also in DLBCL but independently of MCL1, the combination of ABT-199 with the PI3K/mTOR dual inhibitor BEZ235 showed efficacy.56 Interestingly, combining ABT-199 with a number of chemotherapies also showed promising activity in breast cancer, suggesting that BCL-2 alone is also important in the development and maintenance of some solid tumors.77

**Selective targeting of other prosurvival proteins**

Along with selective inhibitors of BCL-2, compounds that target other prosurvival proteins have been developed. These molecules are still in preclinical evaluation, but their increasing availability is proving valuable for investigating the role of each protein individually in healthy and cancerous cells.71,72 The successful development of selective inhibitors of BCL-X, has been reported (WEHI-539, A-1331456, and A-1331852).73-76 Since these compounds have a strong impact on platelet levels, it is likely their use in hematological tumors will require targeting strategies to ameliorate this toxicity. They may find good therapeutic use in solid tumors.72 In addition, selective BCL-X\_ inhibition could potentially re sensitize a large number of cancers to known treatments.73

The prosurvival protein MCL1 has also attracted attention, with several inhibitors published. However, none of these possess the required potency to allow a detailed analysis of efficacy and tolerability.55,77-79 On the one hand, striking data have shown the essential role of MCL1 in hematological cancers, such as AML, multiple myeloma or myc-driven lymphomas.19,29 As mentioned above, it is also a resistance factor for BH3 mimetics targeting other prosurvival proteins such as ABT-199. On the other hand, however, an equal number of stud-
ies based on genetic data suggest that targeting MCL1 may be damaging to a number of tissues in the body, including hematopoietic stem cells,22,23 plasma cells,24 and cardiomyocytes.25 Nevertheless, studies in AML models, have shown that complete elimination of the disease can be achieved with removal of one allele of Mcl1, with no impact on the viability of the animal, suggesting that a therapeutic window may exist.26

Conclusion and outlook
Drug discovery targeting the BCL-2 family of proteins has been, and still is, a significant challenge. Yet, the foresight and perseverance of a few pharmaceutical companies as well as academic institutes have enabled the development of potent drug-like molecules acting on-target. Preclinical data as well as results in patients have clearly validated the initial hypotheses borne out of many years of apoptosis research. These drugs show remarkable efficacy against a range of cancers, some of which had no therapeutic options until recently. Strikingly, the CLL trials have shown that the efficacy of compounds targeting BCL-2 is independent of established predictors of poor prognosis such as 17p deletion, suggesting they will henceforth fill a clinical niche in the greatest need of effective new therapies. The next phase of development will bring more information regarding mechanisms of resistance that may occur and strategies to alleviate them, possibly by combining new agents with other chemotherapies. One of the major lessons of clinical trials with both ABT-263 and ABT-199 is the relative safety of these treatments. Anticipated adverse events (eg., thrombocytopenia) and unexpected adverse events (eg., TLS) have been successfully managed through rational modifications to dosing schedules. Overall, inducers of apoptosis have been well tolerated. The next few years may see clinical trials with BH3 mimetics targeting other prosurvival proteins, mainly BCL-XL and MCL1, both critical proteins in the development of cancer.

References


Although the concept of cancer immunotherapy—leveraging the power of the body’s immune system to fight cancer—is not new, the past few years have seen a surge in the number of positive clinical trials and approvals for new immune-based cancer therapies. Antibody therapies targeted at immune regulatory “checkpoints” and genetically modified T cells delivered via adoptive cell transfer have ushered in a new and exciting period of intense bench and clinical research, after antitumor vaccines failed to fully deliver on their promise as stand-alone agents. Impressive clinical results, especially in tumor histologies not classically associated with response to immunotherapy, have led to renewed enthusiasm for immune-based approaches. Hematological malignancies are greatly benefiting from these novel approaches, being the paradigmatic cancers in which engineered T cells and bispecific antibodies against lineage-specific antigens have demonstrated impressive efficacy. More recently, modulation with anticheckpoint antibodies has yielded impressive results in relapsed, refractory Hodgkin lymphoma, opening the way for the use of these agents in lymphomas and leukemias as monotherapy or in combination with conventional therapies. The new dawn of immunology will permanently change the management of patients with hematological malignancies and allow for precision medicine with an almost infinite number of therapeutic combinations.
lineage-specific molecules that can be targets for substitutive therapies. The present review summarizes questions as well as attempts, failures, and successes of the new dawn for immunology in the treatment of hematological malignancies.

**A brief history: some malignant hematopoietic cells are recognized by the host immune system**

It is striking that acute leukemia was the first human malignancy in which an autologous immune reaction was reported. In the sixties, chemotherapy changed the outcome of pediatric acute lymphoblastic leukemia (ALL). From a rapidly lethal disease, ALL became a pathology in which chemotherapy induced long-term, complete remissions. In the blood of treated patients, malignant lymphoblasts were replaced by normal lymphocytes. In 1969, Wolf Hervé Fridman and François Kourilsky showed that malignant lymphoblasts, taken at the acute phase of the disease and kept frozen, could stimulate the proliferation of autologous lymphocytes purified from the blood of the same patient in complete remission (Figure 1).

This mixed lymphocytes tumor reaction (MLTR), conceived from the model of the mixed lymphocyte reaction between allogeneic cells, was considered a demonstration that patients’ lymphocytes recognize an antigen on their own malignant cells. In a few cases, it was also shown that lymphocytes activated during MLTR, probably T lymphocytes, could kill the autol...
ogous malignant lymphoblasts. In these early years, however, there was no identification of the tumor-associated antigens that triggered the activation of the autologous T cells, and several attempts to vaccinate leukemic patients with killed autologous lymphoblasts were ineffaciously abandoned.

In the 1960s, the most sustained analyses of immune reactions against malignant lymphoid cells were performed in Burkitt lymphoma. Burkitt lymphoma is the consequence of the transformation and immortalization of B lymphocytes by the Epstein Barr virus (EBV). In the Western world, EBV infection is responsible for a very common benign syndrome, infectious mononucleosis, in which infected B lymphocytes are almost totally eradicated by autologous T cells directed towards virus-specific antigens. In Africa, where malarial coinfection results in immunosuppression—and in the Western world in immunodepressed individuals or in anatomical immune sanctuaries, such as the brain—B lymphocytes infected by EBV proliferate due to the absence of T-cell control and form lymphomas. These observations have been considered a clinical argument to support the theory of immune surveillance, but they in fact confirm the role of the immune system in protecting against infections rather than a demonstration of true antitumor immune surveillance. However, EBV-encoded viral antigens represent targets for passive immunotherapies, and there have been successful attempts to treat EBV-positive lymphoma with autologous anti-EBV T cells.

The 1980s witnessed a change of paradigm in the identification of target antigens for immunotherapy of hematological malignancies. Ron Levy realized that malignant B lymphocytes and plasma cells bore idiotypic immunoglobulin (Ig) determinants specific to the malignant cells on their membrane, since the originating normal B-lymphocytic clone did not exist anymore in the patient. These idiotypic determinants therefore represented true tumor-specific antigens and potentially perfect targets for immunotherapy. Levy produced monoclonal antibodies (mAbs) against the idiotypic determinants of circulating Ig produced by the malignant clones of patients with B-cell lymphoma and multiple myeloma and induced long-term remissions in a few patients.

Moreover, attempts to vaccinate patients with B-cell lymphoma against their own idiotypic determinants showed no efficacy and this approach was also abandoned.

Following the same line of thinking, B-cell–differentiation antigens, although not as perfect tumor-specific antigens as idiotypic determinants, appeared to be suitable targets for immunotherapies. Some B-cell–differentiation antigens, like CD19 and CD20, are highly expressed on leukemia and lymphoma cells. This approach turned out to be very efficacious in combination with chemotherapy for the treatment of hematological malignancies: CD20 as a target for mAbs in B-cell lymphoma and chronic lymphocytic leukemia (CLL); and CD19 as a target for bispecific antibodies and T cells engineered with a chimeric antigen receptor (CARTs) in leukemia and myeloma. Based on the relative innocuousness of temporarily depleting the compartment of corresponding normal cells and on the success of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and CARTs, immunotherapy has been permanently added to the list of major therapies for hematological malignancies.

From allogeneic bone marrow transplantation to engineered T cells

The first successful immunotherapy of cancer was in acute leukemia. It was originally not considered immunotherapy, but substitutive therapy; namely the replacement of leukemic bone marrow cells by hematopoietic stem cells from a healthy donor. In 1957, E. Donall Thomas injected six heavily treated leukemic patients with allogeneic bone marrow cells; they all died within 3 months. Six years later, Georges Mathé induced
the first long-term remission in a leukemic patient with an allogenetic bone marrow transplantation. In the 1970s, allogenetic bone marrow transplantation became the reference treatment for acute leukemias and refractory lymphomas in young patients. Stem cells isolated from cord blood or peripheral blood gradually replaced bone marrow. It became evident that the mechanism by which allo-HSCT cured patients was not passive replacement of leukemic stem cells, but an active killing of leukemic blasts by donor T lymphocytes educated in the recipient towards leukemic antigens. This absolute requirement for T cells, activated by recipient antigens, is one of the major hurdles of allo-HSCT, since a graft-versus-leukemia effect is often accompanied by a graft-versus-host reaction due to the recognition of human leukocyte antigen (HLA) antigens by donor T cells. Despite donor selection, it remains a major problem in hematopoietic stem cell transplantation (HSCT). Nevertheless, the latter is still a prominent treatment of acute leukemia and some forms of lymphoma.

Since HSCT demonstrates that properly educated and activated T cells can kill malignant hematopoietic cells with high efficacy, attempts were made to replace allogeneic T cells with autologous T cells in order to prevent a graft-versus-host reaction, while keeping and strengthening the graft-versus-host effect.

In EBV-associated lymphoma, some Hodgkin lymphoma, and some non-Hodgkin lymphoma (NHL), autologous EBV-specific cytotoxic T lymphocytes have been generated and used to treat patients, often in conjunction with HSCT. Long-term remissions have been obtained, but there was no direct proof that they were due to the injected anti-EBV T cells. The revolution in adoptive T-cell therapy of acute leukemia came from the generation of CARTs. The principle of this approach is based on the engineering of T cells taken from the peripheral blood via the introduction of a chimeric receptor, a receptor recognizing a defined antigen linked to a signaling device. All engineered T cells recognize, in addition to their own T-cell receptor, the same tumor-associated antigen. In addition, when they interact with a cell expressing this antigen, they are strongly activated. This technology is a very elegant manner to make all T cells taken from blood specific for a defined tumor-associated antigen. When injected into a patient, they can kill autologous tumor cells with high efficacy. The first use of CARTs was in ovarian cancer, but they really showed high efficacy in leukemia. In 2011, Carl June achieved good clinical responses with a CART that recognized the differentiation antigen CD19 in CLL.

Much more impressively, in ALL treated with CD19 CARTs, up to 100% remission was achieved in pediatric patients and up to 80% in adult patients. Even more importantly, the remissions were long-lasting, with evidence that infused CART levels are maintained in patients for a long period, which may control escaping malignant cells. These successes opened up a new era; many trials are ongoing in ALL, AML, CLL, hairy cell leukemia, and prolymphocytic leukemia. CARTs are being improved with better signaling devices, new chimeric receptors, etc. One potentially game-changing technology has been developed by Cellectics, which engineered T cells not only to express a chimeric anti-CD19 receptor, but also without their own T-cell receptor. Such T cells only recognize CD19—they do not recognize other antigens, in particular, HLA antigens of the recipient. One could therefore use off-the-shelf allogenetic CARTs with no risk of a graft-versus-host reaction. A phase 1 trial is to be started to assess the safety and efficacy of these allogenetic CARTs and their persistence in recipients, since they express HLA antigens and may be rejected. If successful, this approach could really make CARTs pharmaceutical products, with one preparation being taken from the shelf to treat different patients.

From monoclonal to bispecific antibodies

After the first attempts to treat B-cell malignancies with mAbs for the very selective idioype determinants that require the preparation of a mAb for each patient, the field moved successfully towards the use of mAbs directed against B-cell differentiation antigens. The real breakthrough came with rituximab, a mAb directed against the molecule CD20 expressed throughout the B-cell differentiation lineage (with the exception of B-cell precursors and long-lived plasma cells). CD20 is present in high concentrations in most B-cell lymphoma and CLL. It has been approved, in conjunction with chemotherapy, for the treatment of NHL and CLL. Rituximab, a chimeric IgG1 antibody, induces antibody-dependent cellular cytotoxicity via its binding to Fc receptors on natural killer cells and macrophages. It results in the killing, not only of malignant, but also of normal mature B cells, which allows its use in the treatment of certain autoimmune diseases, such as rheumatoid arthritis. This results in a certain degree of immunosuppression, which is easily manageable since T cells are unaffected, as are long-lived plasma cells, which continue to produce antibodies. Eventually, the normal B-cell compartment is reconstituted from unaffected stem cells and precursors in the months following the end of the treatment. An interesting observation was made about the mechanism of action of anti-CD20 antibodies. They not only allow the killing of malignant cells through antibody-dependent cellular cytotoxicity, but also induce a T-cell-mediated antitumor effect via the generation of long-term memory T cells responsible for long-lasting control of the disease, using antigens released by dying cells. Other anti-CD20 mAbs with a higher affinity for Fcy receptors or complement, which are now available, have the goal of improving the therapeutic efficacy of anti-CD20 antibodies.

A myriad of mAbs, directed against differentiation antigens expressed by hematopoietic cells, are being used in trials to treat various types of hematological malignancies. They include: CD19, CD20, CD22, CD33, and CD52 for various types of...
The challenging landscape of hematological malignancies: recent advances

ALL and B-cell lymphomas; CD123 (the IL-3 receptor) or CSF1-receptor for AML; CD274 (PDL-1) for NHL; and anti-CCR4 for cutaneous T-cell lymphomas (reviewed in reference 25). This nonexhaustive list underlines the fact that, in the near future, many tools will become available for selection and association with each other or with other drugs for targeted treatment.

As it became clear that the mechanism of action of antitumor mAbs required two steps—(i) the killing of the tumor cells; and (ii) activation of memory T cells—the concept emerged of directly linking T cells to tumor cells, in order to achieve both steps in one with high efficacy. Bispecific antibodies, with one arm directed towards a tumor antigen and the other towards CD3, a signaling molecule of T-cell receptors, were constructed. This technology, known as BiTE, for bispecific T-cell engager, has, to date, been most successful in the treatment of NHL. Blinatumomab—a bispecific antibody approved for the treatment of ALL, targeting CD19 on B cells and CD3 on T cells—induced impressive tumor responses in relapsed NHL. This technology, known as BiTE, for bispecific T-cell engager, has, to date, been most successful in the treatment of NHL. Blinatumomab—a bispecific antibody approved for the treatment of ALL, targeting CD19 on B cells and CD3 on T cells—induced impressive tumor responses in relapsed NHL.

CD3, a signaling molecule of T-cell receptors, were constructed. This technology, known as BiTE, for bispecific T-cell engager, has, to date, been most successful in the treatment of NHL. Blinatumomab—a bispecific antibody approved for the treatment of ALL, targeting CD19 on B cells and CD3 on T cells—induced impressive tumor responses in relapsed NHL with doses 50 times lower than rituximab. BiTE technology is very efficient. It allows activation of all T cells in the vicinity of targeted malignant cells, providing an off-the-shelf, easy-to-make competitor to CARTs. One hurdle of BiTE technology is that it has a low therapeutic index; the difference in dosage between efficacy and toxicity is small and has to be managed with great care. Another is that it requires administration by continuous infusion. However, BiTEs open up endless possibilities, with the vast array of targetable antigens, the possibility of recruiting cells other than T cells, or bispecific antibodies directed against natural killer cells or macrophage Fc receptors. Many constructs including all the antigens targeted by conventional mAbs are being tested in preclinical and clinical trials in hematological malignancies.

Finally, due to their ability to act as vectors for other molecules, antitumor mAbs have been linked to cytotoxic drugs, toxins, radionuclides, growth factors, and cytokines (Figure 3). Some have been approved, while others are being tested and may be useful for inducing immunogenic cell death of malignant cells as a trigger for a memory T-cell response.

From solid tumors to hematological malignancies: the antitumor antibodies

The most recent improvement in the immunotherapy of hematological malignancies originates from years of research and clinical trials in solid tumors. About a decade ago, tumor immunologists studying the microenvironment of solid tumors discovered that the density of memory T cells in the invasive margin and the center of the tumor was the strongest prognostic factor for a patient's survival. Firstly it was reported in ovarian cancer, then demonstrated and formalized in colorectal cancer and finally extended to most solid tumors.

It was also suggested that antitumor immune reactions could be generated locally in tertiary lymphoid structures, similar to secondary lymphoid organs found in lymph nodes. It became clear that memory T cells, particularly CD8+, when generated in a proper way against tumor-associated antigens, could control tumor growth, spreading, and relapse.

However, in most patients, antitumor T cells are either rare in the microenvironment or ineffective. Studies in chronic infections had revealed that T cells can become exhausted if inefficiently activated by chronic exposure to antigen. They then express on their membrane checkpoint molecules which, upon interaction with their ligands, keep the T cells in a non-responsive state. It rapidly became apparent that in the tumor microenvironment, T cells often express checkpoint molecules to antigen-presenting cells and/or tumor cells expressing their ligands. Both checkpoint-inhibiting and checkpoint-activating molecules exist (Figure 4).

It is to the merit of James P. Allison that he developed the concept of blocking the interaction with its ligand of the checkpoint inhibitor CTLA-4, liberating T cells to efficiently kill target cells they recognized. A pivotal trial was published in 2010 with ipilimumab, an anti-CTLA-4 antibody, which induced long-term survival in metastatic melanoma patients and led to approval of the mAb. It opened a new field with a change of paradigm where it is not the tumor cells that are therapeutically targeted, but the immune cells. A second checkpoint molecule, which shows even greater promise, is PD-1, which is induced by T-cell activation. Activated T cells produce IFN-γ, which induces or increases the PD-1 ligands PDL-1 and PDL-2 on adjacent tumor cells, forming a shield against immune attack. Disrupting this interaction using anti-PD-1 or anti-PDL-1 antibodies yielded impressive tumor responses and increased patient survival in melanoma, lung cancer, renal cell cancer, and bladder cancer, in which their use has
A new dawn for immunology in the treatment of hematological malignancies – Fridman

The challenging landscape of hematological malignancies: Recent advances

Conclusions

The immunotherapy tool box for hematological malignancies is rich and diverse. Successors of HSCT, the use of CAR-Ts, tumor-targeted monoclonal or bispecific antibodies, and most recently immunomodulatory checkpoint inhibitor antibodies have changed the treatment paradigm of hematological malignancies. The management of the diseases will be changed in a lasting way by the combination of these multiple approaches with conventional and targeted therapies, creating a new dawn for immunology and oncohematology.

References


Keywords: history of immunotherapy; immune surveillance; cell therapies; immune checkpoints; antibody therapies
The versatility of the DART® platform for multiple therapeutic applications

by G. R. Chichili, P. A. Moore, S. Johnson and E. Bonvini, USA

Bispecific antibodies are designed to simultaneously recognize two different epitopes. The dual specificity opens a wide range of applications for bispecific antibodies, including redirecting immune effector cell cytotoxicity to tumors, blocking two different signaling pathways simultaneously on the same cell, and dual targeting of different disease mediators, such as pathogens or soluble inflammatory molecules. The DART® (dual-affinity re-targeting) platform is a flexible and robust bispecific antibody platform that combines Fv regions that simultaneously bind two target antigens. The modular architecture of the DART platform enables the reconfiguration and design of molecules with the desired specificity pair, valency, and half-life for a variety of therapeutic modalities. There are currently five DART molecules in clinical development for the treatment of cancers, one molecule for autoimmune diseases, and several additional DART molecules in development for additional cancer indications and infectious diseases. In this monograph, we discuss several examples of DART proteins designed for redirecting T-cell cytotoxicity to tumor cells for both hematological malignancies and solid tumors, modulation of receptor signaling through linking inhibitory and activation pathways in B cells, and targeting multiple epitopes on viruses for enhanced neutralization.
able region light (VL) and heavy (VH) chains specific for two different antigens arranged in a bispecific format with the order VL (1)-VH (2) and VL (2)-VH (1). Short linker sequences between the VL and VH segments promote a posttranslational "diabody"-type association. E- and K-coils promote the desired heterodimeric structure, with the association of the two chains stabilized through covalent carboxy-terminal disulfide linkage. Other bispecific strategies, such as fragment-based and Ig-like modalities, often rely on noncovalently linked single-chain fragment variable or diabody structures to maintain the integrity of the binding domain; because the strength of the association between the VL and VH is quite variable, with variable equilibrium between the associated and nonassociated forms, the stability of these molecules in solution and under conditions of use have been highly unpredictable. The DART platform overcomes the limitations of these bispecific formats, and DART molecules can be produced easily in mammalian expression systems and without the need for a refolding process. DART molecules have been shown to be exceptionally stable, both under storage and use conditions, such as in human serum at 37°C for an extended period of time. Unlike bispecific T-cell engager (BiTE®), an alternate format of single-chain fragment variable bispecific molecules, the interchain linkers and the covalent linkage between the two DART chains limit the freedom of the antigen-binding domains. The resolved crystal structure of DART molecules has been shown to be a tightly assembled compact spherical structure compared with other diabodies. Therefore, DART molecules are structurally more favorable to drive stable association between target and effector cells. In a study that compared a CD19 × CD3 DART molecule to an equivalent BiTE, it was demonstrated that the DART molecule was more potent than the BiTE in killing B-cell lymphoma cells.

The flexibility of the DART platform allows for the generation of stable bispecific molecules by easily swapping different antigen-binding domains with consistent chain pairing and predictable antigen recognition. The modular architecture of the platform also allows the engineering of multiple variants with different numbers of antigen-binding sites and valency for each antigen, spatial relationships between different binding sites, and pharmacokinetic properties. Figure 1 shows examples of DART molecules that are currently in development for a range of different applications. This format diversity provides the ability to tailor DART molecule design to match desired mechanisms of action and intended clinical application. We discuss here DART molecules that have been developed for three different mechanisms of action: redirected T-cell cytolysis for tumor cell killing; modulation of receptor signaling; and targeting of multiple epitopes on viruses for enhanced neutralization and clearance.

### DART molecules for redirecting T-cell-mediated cytolysis

Redirected target-cell killing by immune effector cells is the most widely used approach of bispecific antibodies in cancer immunotherapy, where bispecific antibodies are designed to simultaneously bind to a cytotoxic effector cell, via a receptor like CD3 or CD16, and a target cell expressing a tumor-specific antigen. This bispecific antibody–mediated coassociation of effector and target cells results in the formation of a synapse between the two cells resulting in activation of the cytotoxic pathway in effector cells and tumor cell death (Figure 2). T lymphocytes are major components of the adaptive immune system that are widely distributed within tissues and the tumor microenvironment. They play a central role in
cell-mediated immunity and can mediate long-lived, antigen-specific, effector and immune-memory responses. T cells are activated when they recognize cognate peptide antigens in the context of major histocompatibility complex molecules, which are expressed on the surface of antigen-presenting cells. Once activated, they rapidly proliferate and assist in the active immune response, such as target-cell killing and secretion of cytokines that regulate other immune cells. CD3 is an invariant complex of proteins required for expression of and signaling through the antigen-specific T-cell receptor on T cells. T lymphocytes also play an important role in the immune response against cancer; however, tumor-specific T-cell responses are limited by immune escape mechanisms utilized by tumor cells. CD3-based bispecific antibodies, such as DART molecules, can engage T cells with tumor cells; this process is accompanied by the formation of an artificial transient cytolytic synapse between the T cell and the targeted tumor cell, eventually resulting in activation and proliferation of T cells and to tumor-cell lysis (Figure 2).

Both the anti-CD123 and anti-CD3 components of MGD006 are crossreactive with the corresponding cynomolgus monkey antigens, with affinities similar to those for the human antigens; MGD006 can redirect T cells from either species to kill CD123-expressing cells. Monkeys infused with escalating doses of MGD006 over a period of 4 weeks showed depletion of circulating CD123+ cells throughout the 4-week treatment period. T-cell redistribution was observed after each MGD006 dose. However, T cells from treated monkeys exhibited efficient ex vivo redirected target-cell killing, indicating no functional exhaustion. Transient release of cytokines, particularly IL-6, was observed following the first MGD006 infusion, but not after subsequent administrations even when the dose was escalated. Near complete elimination of CD123+ progenitors in the bone marrow was also observed with a reversible decrease in red cell mass. No significant changes in circulating platelets or neutrophil levels were observed. Currently, MGD006 is in a phase 1 study in patients with relapsed and refractory AML or intermediate-2/high-risk myelodysplastic syndrome (NCT02152956).
The versatility of the DART® platform for multiple therapeutic applications – Bonvini and others

PF-06671008 (P-cadherin × CD3 DART molecule)
PF-06671008 is a long-acting P-cadherin × CD3 DART that engages P-cadherin–expressing cells and T cells. Upregulation of P-cadherin has been reported in various tumors, including breast, gastric, endometrial, colorectal, and pancreatic cancers; upregulation is correlated with poor survival of breast cancer patients. P-cadherin overexpression has also been shown to correlate with increased tumor cell motility and invasiveness. PF-06671008, a P-cadherin × CD3 DART molecule, demonstrated potent cell-mediated killing activity against P-cadherin–expressing tumor cells and potent in vivo efficacy in colorectal cancer/PBMC comix xenograft mouse models. PF-06671008 is currently being investigated in a phase 1 study in patients with solid tumors expressing P-cadherin (NCT02659631).

MGD007 (gpA33 × CD3 DART molecule)
MGD007 is another Fc-bearing DART protein that binds glycoprotein A33 (gpA33) and CD3 simultaneously, gpA33 is a lineage-specific antigen expressed in the intestinal epithelium and is present in >95% of primary and metastatic human colorectal cancers. MGD007 was designed to redirect the killing function of CD3-expressing effector T cells to target cells expressing gpA33. In vitro studies with MGD007 have demonstrated high-affinity binding to both human and monkey CD3 and gpA33. MGD007 mediates potent redirected T-cell killing of gpA33-expressing colorectal cancer target cells, with EC50 values as low as ≈1 ng/mL in assays employing purified human T cells as effector cells. Consistent with its design, upon coengagement of gpA33-expressing target cells and T cells, MGD007 mediates dose-dependent T-cell activation, T-cell proliferation, and upregulation of granzyme B and perforin. As with the other DART molecules, the biological activity of MGD007 is exquisitely dependent on coengagement of both gpA33- and CD3-expressing cells. MGD007 inhibited tumor growth in mice implanted with human colorectal cancer cells in the presence of activated human T cells. In cynomolgus monkeys, 4 weekly doses of up to 200 g/kg were well tolerated, with prolonged pharmacokinetics consistent with those of an Fc-containing molecule supporting convenient dosing. Currently MGD007 is being evaluated in a phase 1 trial in patients with relapsed colorectal cancer (NCT02248805).

MGD014 (HIVenv × CD3 DART molecule)
Another application for CD3-based DART molecules is the elimination of cells that act as HIV reservoirs in chronically infected patients. In chronically infected patients treated with combination antiretroviral therapy, HIV reservoirs and production of viral antigens are not completely eliminated. The HIV envelope protein (env) is a highly specific viral target for therapeutic elimination of persistent HIV reservoirs via antibody-mediated cell cytotoxicity. HIVenv × CD3 DART molecules target T cells to envelope proteins of HIV-infected cells; they are engineered from antibodies known to recognize diverse env epitopes (with or without broadly neutralizing activity) and mediate potent cytotoxic T lymphocyte–dependent killing of quiescent primary CD4 T cells infected with diverse HIV isolates. Similar killing activity was also observed with DART molecules structurally modified for extended circulating half-life. Using lymphocytes from patients on suppressive antiretroviral therapy, it was demonstrated that HIVenv × CD3 DART molecules mediated clearance of CD4+ T cells that were superinfected with a laboratory HIV-1 strain (JR-CSF) or infected with autologous reservoir viruses isolated from HIV-infected patients. Also, HIVenv × CD3 DART molecules mediated clearance of HIV-infected resting CD4+ T-cell cultures following induction of latent virus, without inducing cell-to-cell virus spread in resting or activated CD4 T-cell cultures. Combined with HIV latency-reversing agents, HIVenv × CD3 DART proteins have the potential to be effective immunotherapeutic agents for clearing latent HIV-1 reservoirs in HIV-infected individuals.

DART molecules for modulation of receptor signaling
Activation-inhibition coupling, the pairing of a positive signal with an inhibitory loop, controls the magnitude and duration of many biologic processes. A CD32B × CD79B DART molecule (MGD010) was designed to specifically engage CD32B and CD79B to exploit the physiologic receptor Fc RIIb (CD32B) inhibitory coupling mechanism to control B-cell activation. The CD32B molecule is a transmembrane inhibitory receptor expressed on B cells and other immune cells, such as macrophages, neutrophils, and mast cells. CD79B, the immunoglobulin-associated subunit of the B-cell receptor (BCR) complex, is an essential signal transduction component of the BCR that is expressed exclusively on B cells. CD32B × CD79B DART molecules simultaneously engage CD32B and CD79B in preferential cis-binding mode on B lymphocytes. Coligation of CD32B and CD79B triggers CD32B-coupled immunoreceptor tyrosine-based inhibitory motif (ITIM) signaling, which decreases antigen-mediated B-cell activation without broad B-cell depletion. MGD010 inhibits normal human B-cell proliferation induced by BCR stimulation even in the presence of B-cell growth factors, such as BlyS. MGD010 also reduced calcium flux in response to BCR stimulation in both normal naïve and memory B cells. Importantly, MGD010 inhibited BCR-induced B-cell proliferation in systemic lupus erythe-
mariosus patient samples, providing evidence of activity in the setting of pathological B cells from patients with autoimmune disease. By ligating each component in an essentially monovalent fashion, MGDO10 exerts its inhibitory activity only in the context of antigen-receptor signaling and has no intrinsic activation properties. The effects of MGDO10 on human B-cell function and the pathophysiology of autoimmune disease were confirmed in vivo using humanized mouse models. MGDO10 is currently under evaluation in a phase 1 clinical trial in healthy volunteers (NCT02376036).

**DART molecules for virus neutralization**

Bispecific multivalent DART molecules have also been successfully used for neutralizing viruses. Two examples of such applications include DART molecules directed toward broad neutralization of influenza (H5N1 × H5N1 DART molecules) and dengue (DENV × DENV DART molecules), engineered through the combination of two highly efficacious virus-neutralizing antibodies for the respective viruses. These DART molecules were engineered with human IgG constant regions (C1, C2, and C3), thus, two DART molecules are joined into a single molecule in a tetravalent, bispecific format termed “Ig-DART”.

While vaccines are available for the highly pathogenic H5N1 avian influenza viruses, other approaches are required for people that typically respond poorly to vaccination, such as the elderly and the immunocompromised. The H5N1 × H5N1 DART molecule is a bispecific Fc-bearing DART protein engineered from combining two neutralizing mAbs that target variants of the globular head of the hemagglutinin antigen.10 The neutralization activity of the H5N1 × H5N1 DART protein against H5N1 flu viruses was consistent with that of the parental antibodies. Interestingly, the H5N1 × H5N1 DART molecule also showed neutralization activity against a virus strain that was not neutralized by either of the parental antibodies, suggesting an increased breadth of specificity for the combination. The H5N1 × H5N1 DART molecule provided 100% protection against virus challenge in mice and ferrets, when it was used as either a prophylactic or therapeutic agent against antigenically drifted virus strains. Therefore, therapy consisting of the H5N1 × H5N1 DART molecule alone is likely to be as efficacious as a cocktail containing both mAbs and may be more broadly active against antigenically diverse H5N1 virus strains.

The DENV × DENV DART molecule combines the specificity of mAb E60, a crossreactive antibody that binds the DIII-FL protein, and mAb 4E11, a complex-specific antibody that binds the A-strand epitope on the DIII protein of virion.21 In vitro, the neutralization potential of the DENV × DENV DART molecule was modestly improved compared to the mixture of the two parental mAbs. In mice, administration 48 hours after infection protected against lethal disease in a manner similar to the cocktail of parental antibodies.48 The DENV × DENV DART may have advantages over traditional mAb combination therapy because, as a single product, it carries simplified regulatory requirements and reduced cost of goods compared to mAb combinations.

**Conclusions and future directions**

The flexibility, modular nature, as well as the favorable biophysical and functional properties of the DART format make it an ideal platform for next-generation bispecific molecules, with the potential for application in multiple therapeutic areas. Such versatility is highlighted by the number of DART molecules currently in clinical development for the treatment of cancers and autoimmune diseases. Examples of potential uses include engagement of cells other than T lymphocytes as effector cells, by switching the effector arm in the DART molecule to another activating receptor, thus widening the spectrum of strategies to recruit, boost, modulate, or activate the immune system in tumor settings. Other areas where the DART platform would prove extremely useful include improved target-cell selectivity via dual antigen (biepitopic) recognition or the targeting of multiple checkpoint molecules on immune cells, providing opportunities for single-molecule combinatorial strategies of checkpoint inhibition. Dual-targeting strategies via bispecific antibodies will bring innovative solutions that are poised to deliver exciting clinical opportunities in the near future.

DART is a registered trademark of MacroGenics. BiTE is a registered trademark of Amgen.

**References**

Keywords: dual-affinity re-targeting; bispecific antibody; epitope; cytolysis; receptor signaling; virus neutralization
Building on the foundations of chemotherapy’s success: the place of novel treatments in lymphoma therapy

by M. Nicolosi, A. Castellino, and U. Vitolo, Italy

Lymphomas are hematological malignancies treated with schemes of standard chemotherapy. Several studies have demonstrated that there is a need to ameliorate first-line treatment to improve remission rate, as in diffuse large B-cell lymphoma and Hodgkin lymphoma, and to reduce the relapse rate, as in indolent or mantle cell lymphoma. Moreover, the treatment of relapsed/refractory patients is still unsatisfactory. Mechanisms of cancer immune-surveillance have been largely established in solid tumors, and recent data have shown analogous scenarios in lymphoproliferative disorders. On the basis of this promise, several new “chemotherapy free” strategies have been developed, introducing novel drugs and new molecules such as monoclonal antibodies, checkpoint inhibitors, immunomodulatory drugs, phosphoinositide 3-kinase inhibitors, and Bruton’s tyrosine kinase inhibitors. In this review, we want to analyze the different strategies of treatment with a focus on the setting of new drugs and new molecules.

Hodgkin lymphomas

The 2008 World Health Organization classification recognized two major categories of lymphoma: non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma. The most common aggressive histotype of NHLs is diffuse large B-cell lymphoma (DLBCL).1 Indolent forms include follicular lymphoma, chronic lymphocytic leukemia, some cases of mantle cell lymphoma, marginal zone lymphoma, and splenic marginal zone lymphoma. The present review focuses on the standard of care of NHLs and Hodgkin disease as well as the most recent achievements in this broad and rapidly evolving field.

Hodgkin lymphomas

The standard treatment for limited or intermediate-stage patients with Hodgkin lymphoma is a combined modality with two to four cycles of adriamycin/bleomycin/vinblastine/dacarbazine (ABVD) followed by involved-field radiation therapy.2 However, the current radiation therapy guidelines of the International Lymphoma Radiation Oncology Group recommend involved-site radiation therapy.3 Advanced-stage Hodgkin lymphoma is usually treated with chemotherapy alone and ABVD represents the standard regimen, although BEACOPP (bleomycin/etoposide/Adriamycin [doxorubicin]/cyclophosphamide/Vincristine/Procarbazine/prednisone) is also a reasonable option, but with more side effects. Additional radiation therapy is confined to patients with residual disease after chemotherapy or as a consolidation for bulky areas.4 Retrospective analyses have indicated that early interin

Address for correspondence:
Dr Maura Nicolosi, Dipartimento di Oncologia ed Ematologia, A. O. Città della Salute e della Scienze di Torino, C.so Bramante 88/90, 10126 Torino, Italy (email: manicolosi@cittadellasalute.to.it)

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Figure 1. B-cell receptor cascade and target therapy.

Sites in the B-cell receptor signaling pathway at which ibrutinib, idelalisib, and fostamatinib act as inhibitors. Panel A shows that ibrutinib inhibits the phosphorylation of Btk thus preventing activation of PLC-γ2. Panel B shows that fostamatinib inhibits Syk kinase activity as well as that of Lyn. Idelalisib inhibits PKC, which limits the formation of PIP3. This blocks membrane interactions of signaling proteins such as PDK1, Atk, and Btk.

Abbreviations: Ag, antigen; Atk, protein kinase B; Btk, Bruton’s tyrosine kinase; BCR, B-cell receptor; PLC-γ2, phospholipase C-γ2; PI3K, phosphatidylinositol 3-kinase; PKD, phosphatidylinositol kinase 3; PIP3, phosphatidylinositol 3,4,5-triphosphate; PIP2, phosphatidylinositol 4,5-bisphosphate.


positional emission tomography (PET) might be a good predictor for treatment failure in patients with advanced Hodgkin lymphoma receiving ABVD chemotherapy. Recently, trials aimed at guiding treatment on the basis of early interim PET have shown that in patients who remained positive at interim PET changing to a more intensive treatment improved outcome. For most patients with refractory or relapsed Hodgkin lymphoma, the treatment of choice consists of high-dose chemotherapy followed by autologous stem cell transplantation (ASCT).

Non-Hodgkin lymphomas

- **Diffuse large B-cell lymphoma (DLBCL)**
  - Rituximab with cyclophosphamide/hydroxydaunorubicin (doxorubicin)/Oncovin (vincristine)/prednisone (R-CHOP) every 21 days, is now the current standard treatment for most patients with newly-diagnosed DLBCL. The complete remission rate of newly diagnosed DLBCL is approximately 65% to 75% with R-CHOP. An alternative regimen for first-line treatment is DA-EPOCH-R (dose-adjusted etoposide/prednisone/Oncovin (vincristine)/cyclophosphamide/hydroxydaunorubicin (doxorubicin)—rituximab). DLBCL subtypes respond differently to standard R-CHOP chemotherapy. The activated B-cell DLBCL subtype is associated with a poorer prognosis when treated with R-CHOP only. In contrast, patients with germinal center B-cell DLBCL and primary mediastinal B-cell lymphoma have a better outcome. Patients who have c-MYC/BCL-2 or c-MYC/BCL-6 double-hit or double expression of c-MYC and BCL-2 proteins have a poorer outcome because of their aggressive clinical course. Relapsed or refractory patients have a poor prognosis, and standard therapy is high-dose chemotherapy combined with rituximab, followed by ASCT. Various salvage regimens are available in relapsed or refractory DLBCL. However, salvage therapy and transplant conditioning regimens are still suboptimal.

- **Mantle cell lymphoma**
  - Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma. Stages I and II are generally associated with an indolent course. A current recommendation includes shortened chemotherapy induction followed by consolidating radiation. Treatment for young patients with stages III or IV is intensive immunochemotherapy with ASCT along with maintenance therapy. There are different schemes, such as R-hyper-CVAD (rituximab plus fractionated cyclophosphamide, vincristine, Adriamycin [doxorubicin], and dexamethasone) or R-CHOP alternating with R-DHAP (rituximab plus dexamethasone, high-dose ara-C [cytarabine], platinum [cisplatin]) + high-dose therapy/ASCT. Both of them induce a high complete remission rate, but relapses occur in 30% to 40% of patients.
In fit elderly patients, R-CHOP or rituximab/bendamustine can be used with good control of the disease, but few chances of a cure. Rituximab maintenance has been proven to improve progression-free survival and overall survival in elderly patients. Nevertheless, the management of relapse and progression is still an unmet medical need with few standard options for these patients.

**Follicular lymphoma**

Follicular lymphoma is an indolent NHL. The addition of the anti-CD20 monoclonal antibody to traditional chemotherapy prolongs survival of such patients. In patients with limited nonbulky stages I or II, involved-field radiation therapy (24 Gy) is preferred. In selected cases, watchful waiting or rituximab monotherapy may be considered. R-CHOP or rituximab/bendamustine is the standard of care in advanced-stage disease. A brief course of chemoinmunotherapy with rituximab followed by consolidation with rituximab is an alternative in elderly patients. Rituximab maintenance for 2 years improves progression-free survival. High-dose chemotherapy plus ASCT should be considered, especially in patients with short-lived first remissions after rituximab-containing regimens.

**Novel drugs**

Accordingly, for different lymphoma subtypes, there is a need to ameliorate treatment both first-line to improve complete remission rate, as in DLBCL, for example, or to reduce the relapse rate, as in indolent lymphoma or MCL. Moreover, the treatment of relapsed/refractory patients is still unsatisfactory. In solid tumors, mechanisms of cancer immune-surveillance have been largely established, and recent data have shown analogous scenarios in lymphoproliferative disorders. In B-cell lymphomas, neoplastic cells adopt a plethora of strategies to escape immune surveillance, including downregulating costimulatory molecules or effecting changes in the surrounding microenvironment. A better knowledge of specific molecular pathways involved in lymphoma pathogenesis and cell survival has led to the development of specific inhibitors that overcome resistance or refractoriness to standard treatment (Figure 1).

**Naked monoclonal antibodies**

The CD20 molecule is a phosphoprotein that is highly expressed on the surface of B cells and that plays a fundamental role in the B cell, making it an attractive target for a specific immunotherapeutic approach. Rituximab was, in 1997, the first anti-CD20 chimeric monoclonal antibody (mAb) to be approved for the treatment of patients affected by B-cell NHL, such as follicular lymphoma, DLBCL, MCL, and chronic lymphocytic leukemia (CLL). Rituximab represents a cornerstone in the treatment of B-cell malignancies, and new humanized anti-CD20 mAbs have been developed in the last decades (Figure 2).

**Ofatumumab**

Ofatumumab is a second-generation, type I, fully human anti-CD20 IgG1 mAb with a higher complement-dependent cytotoxicity in malignant B cells with low CD20 expression. Ofatumumab has been approved in patients affected by relapsed or refractory B-cell CLL, showing an overall response rate (ORR) of 58%. In a recent randomized phase 3 trial that compared ofatumumab versus physicians’ best choice treatment...
in patients affected by fludarabine-refractory B-cell CLL,\textsuperscript{31} median progression-free survival and time to next therapy were significantly longer with ofatumumab (7.0 vs 4.5 months and 11.5 vs 6.5 months, respectively).

\textbf{Obinutuzumab (GA101)}

Obinutuzumab (GA101) is a third-generation type II glycoengineered, humanized anti-CD20 mAb designed to have enhanced activity compared with other available anti-CD20 antibodies. GA101 in monotherapy was first investigated in a phase 1/2 multicenter trial in a cohort of patients with relapsed or refractory NHL or B-cell CLL. ORR was 56%, with a median progression-free survival of 11.8 months.\textsuperscript{32} GA101 has also been explored in a phase 1b study in combination with chemotherapy regimens in patients affected by relapsed or refractory follicular lymphoma, with an ORR higher than 90%.\textsuperscript{33}

Sehn et al\textsuperscript{34} presented the data of a large randomized phase 3 trial that compared obinutuzumab plus bendamustine versus bendamustine monotherapy in 396 patients affected by rituximab-refractory indolent NHL. Progression-free survival was significantly longer with obinutuzumab plus bendamustine (median not reached) than with bendamustine monotherapy (14.9 months), suggesting that obinutuzumab may be effective in rituximab-refractory patients (Table I).\textsuperscript{32,35-37}

\textbf{Drug-conjugated monoclonal antibodies}

In order to improve efficacy, antibodies have been coupled to highly potent cytotoxic drugs or toxins.

\textbf{Polatuzumab vedotin}

Polatuzumab vedotin is an antibody–drug conjugate containing an anti-CD79B monoclonal antibody conjugated to the microtubule-disrupting agent monomethyl auristatin E. A phase 1 multicenter study, aimed at assessing the safety and clinical activity of polatuzumab vedotin in 95 patients with relapsed or refractory NHL and B-cell CLL, was recently published.\textsuperscript{38} The most common grade 3 or 4 toxicities were neutropenia, anemia, and peripheral sensory neuropathy, with an ORR of 54.7% in the single-agent polatuzumab vedotin arm and 77.7% in the polatuzumab vedotin plus rituximab arm. No responses were noted in patients with CLL. Further larger studies with this molecule in relapsed/refractory NHL are ongoing.

\textbf{Brentuximab vedotin}

Brentuximab vedotin is a chimeric mAb against human CD30 coupled to monomethyl auristatin E. Brentuximab vedotin has been employed in CD30+ Hodgkin lymphoma in a T-cell and primary mediastinal B-cell lymphoma setting. A phase 1 trial on brentuximab vedotin in Hodgkin lymphoma was conducted in 45 patients.\textsuperscript{39} Subsequently, a multicenter phase 2 study investigated the efficacy of brentuximab vedotin in 105 advanced Hodgkin lymphoma patients who had failed ASCT, with an ORR of 75% and complete remission in 34%.\textsuperscript{40} Based on these data, brentuximab vedotin had accelerated approval in the USA and Europe. Additional trials were designed to investigate the use of brentuximab vedotin in different settings of Hodgkin lymphoma: as consolidation after ASCT\textsuperscript{41} or in association with standard treatment in first-line patients.

\begin{table}
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
\textbf{Study} & \textbf{Design and size} & \textbf{Patient population} & \textbf{Treatment} & \textbf{Clinical activity} & \textbf{Duration of activity} \\
\hline
GAUGIN\textsuperscript{32} & Phase 2, n=40 & Relapsed/refractory indolent NHL & Ob single-agent, 2-dose level & ORR & - \\
& & & & High-dose, 55% Low-dose, 17% & \\
\hline
GAUGIN\textsuperscript{2\textsuperscript{36}} & Phase 2, n=40 & Relapsed/refractory aggressive NHL (MCL and DLBCL) & Ob single-agent, 2-dose level & ORR & - \\
& & & & High-dose, 37% Low-dose, 24% & \\
\hline
GAUDI\textsuperscript{36} & Phase 2, n=56 & Relapsed/refractory FL & Ob at dose levels and systemic chemotherapy (CHOP or FC) & ORR & Not reported \\
& & & & Ob-CHOP, 96% Ob-FC, 93% Ob-CHOP, 29% Ob-FC, 50% & \\
\hline
CLL\textsuperscript{11\textsuperscript{37}} & Phase 3, n=781 & Previously untreated elderly CLL patients & CI alone vs R-CI vs Ob-CI & ORR & \\
& & & & CI, 30.2% Ob-CI, 75.5% R-CI, 65.9% & \\
& & & & CI, 0% Ob-CI, 22.2% R-CI, 8.3% & \\
& & & & PFS & \\
& & & & CI, 11.1 m Ob-CI, 26.7 m R-CI, 16.3 m OS & \\
& & & & No differences so far & \\
\hline
\end{tabular}
\caption{Summary of obinutuzumab clinical studies in lymphoid malignancies.}
\textbf{Abbreviations:} CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CI, chlorambucil; CLL, chronic lymphocytic leukemia; CRR, complete response rate; DLBCL, diffuse large B-cell lymphoma; FC, fludarabine and cyclophosphamide; FL, follicular lymphoma; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; Ob, obinutuzumab; ORR, overall response rate; OS, overall survival; m, months; PFS, progression-free survival; R, rituximab. Data from reference 29.}
\end{table}
Another important mechanism that could be altered in lymphomas is the control of immune checkpoints to prevent the active evasion of immune surveillance. One such example is the overexpression of ligands for programmed cell death protein 1 receptors (PD-L1) on the surface of some lymphomas, such as Hodgkin lymphoma. Novel agents that interfere with this interaction have been developed to restore T-cell cytotoxicity against tumor cells. Nivolumab is a mAb that binds to the programmed cell death protein 1 (PD-1) receptor and prevents its interaction with PD-L1. In this way, it overcomes immune tolerance induced by cancer cells. A phase 1 trial in heavily pretreated Hodgkin lymphoma showed an ORR of 87%, with complete remission in 17%, with a durable response. On the basis of these results, a registrational phase 2 trial is ongoing. Pembrolizumab is another mAb that targets PD-1 receptors. A phase 1b study has been conducted in pretreated Hodgkin lymphoma. ORR was 65%, complete remission was 16%, stable disease was 23%; overall, 87% of patients had a clinical benefit. These results suggested that PD-1 inhibition is a safe strategy, with strong efficacy in heavily pretreated Hodgkin lymphoma. Further studies are needed to explore the role of these agents in first-line Hodgkin lymphoma and in other lymphoma setting (Figure 3).

Checkpoint inhibitors

- **Anti-PD-1/PD-L1**

Among new drugs used in B-cell lymphoma, immunomodulatory drugs, such as lenalidomide, have an important role. Lenalidomide has several different modes of action, including direct cytotoxicity, restoration of immune synapsis, and microenvironment immunomodulation enhancing natural-killer and T-cell activity. It has been studied in several series of relapsed or refractory DLBCL, showing promising data mostly in nongermineral center origin series. On this basis, two prospective multicenter phase 1/2 studies were conducted to investigate the efficacy and safety of adding lenalidomide at different doses (15 or 25 mg) to R-CHOP every 21 days in newly diagnosed DLBCL patients. Both studies reported a good safety profile (even in the elderly) and prolonged progression-free survival, especially in the nongermineral center cell subtype. In the Italian REAL07 study, with an ORR of 92% and complete remission in 86%, 2-year progression-free survival and 2-year overall survival were 80% and 92%, respectively. Similar results were observed in a Mayo Clinic study (Figure 4, page 368). Based on these results, a randomized multicenter phase 3 trial is ongoing (R-CHOP + lenalidomide) in patients with activated B-cell DLBCL, determined by gene expression profiling with nanostring technology (ROBUST trial [NCT02285062]). Lenalidomide has also been studied in other NHLs, such as MCL, where it has demonstrated a good safety profile and efficacy. 

Leonard et al conducted a randomized trial of lenalidomide monotherapy and lenalidomide plus rituximab versus rituximab monotherapy in patients with relapsed follicular lymphoma; the arm with rituximab alone was closed earlier for inferiority. In the lenalidomide monotherapy arm, ORR was 51.1%, with complete remission in 13.3%. In the combination arm, the ORR was higher (72.7%), with complete remission in 36.4%. Median event-free survival was 1.2 years in the lenalidomide arm alone versus 2 years in the combination arm (P=0.008). There was no difference in overall survival. Fowler et al conducted a phase 2 study using the association of lenalidomide plus rituximab as a first-line strategy in patients with indolent NHL. ORR was 98% in follicular lymphoma, with complete remission in 87%, documented by PET; 3-year progression-free survival was 81%. This result has been confirmed in other series. On the basis of these findings, a randomized multicenter phase 3 trial (RELEVANCE) is ongoing to compare the combination of rituximab plus chemotherapy followed by two years of maintenance rituximab versus free chemotherapy treatment with lenalidomide plus rituximab, followed by rituximab maintenance.

Immunomodulatory drugs

- **Lenalidomide**

Among new drugs used in B-cell lymphoma, immunomodulatory drugs, such as lenalidomide, have an important role. Lenalidomide has several different modes of action, including direct cytotoxicity, restoration of immune synapsis, and microenvironment immunomodulation enhancing natural-killer and T-cell activity. It has been studied in several series of relapsed or refractory DLBCL, showing promising data mostly in nongermineral center origin series. On this basis, two prospective multicenter phase 1/2 studies were conducted to investigate the efficacy and safety of adding lenalidomide at different doses (15 or 25 mg) to R-CHOP every 21 days in newly diagnosed DLBCL patients. Both studies reported a good safety profile (even in the elderly) and prolonged progression-free survival, especially in the nongermineral center cell subtype. In the Italian REAL07 study, with an ORR of 92% and complete remission in 86%, 2-year progression-free survival and 2-year overall survival were 80% and 92%, respectively. Similar results were observed in a Mayo Clinic study (Figure 4, page 368). Based on these results, a randomized multicenter phase 3 trial is ongoing (R-CHOP + lenalidomide) in patients with activated B-cell DLBCL, determined by gene expression profiling with nanostring technology (ROBUST trial [NCT02285062]). Lenalidomide has also been studied in other NHLs, such as MCL, where it has demonstrated a good safety profile and efficacy. 

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Histone deacetylase inhibitors

Histone deacetylase inhibitors modulate gene expression in a wide variety of malignancies, increasing the expression of cell cycle regulators, differentiation genes, tumor antigens, and genes encoding proapoptotic proteins. A phase 2 trial with vorinostat, an oral histone deacetylase inhibitor, in relapsed or refractory follicular lymphoma, marginal zone lymphoma, and MCL reported an ORR of 29%, with complete remission in 14.5%. A similar phase 2 trial with the combination of vorinostat and rituximab is underway. Vorinostat is also being studied in combinations with traditional chemotherapy in DLBCL.
Other histone deacetylase inhibitors that have been investigated in T-cell lymphoma are romidepsin and belinostat, with interesting preliminary results.

- **Anti-BCL-2 drugs**
  Overexpression of BCL-2 is common in NHL and associated with poor response to therapy and shorter survival. Modulation of BCL-2 may enhance the efficacy of conventional chemotherapy.

  ABT-199 (venetoclax) represents a first-in-class, selective, oral BCL-2 inhibitor, which was first studied in CLL, where it showed an ORR >80%. An ORR of 48% was obtained with ABT-199 in a phase 1 trial of ABT-199 in relapsed or refractory NHL. ABT-199 has also been studied in combination with other agents. A phase 3 trial comparing ABT-199 plus rituximab vs bendamustine plus rituximab in previously treated CLL is ongoing. A phase 1 study of ABT-199 in combination with rituximab/bendamustine showed that ORR was 61.5% in relapsed or refractory NHL, 73% in follicular lymphoma, and 37.5% in DLBCL patients. The phase 2 CONTRALTO trial in relapsed or refractory follicular lymphoma is ongoing.

- **Bruton’s tyrosine kinase inhibitor**
  There have been interesting results with ibrutinib, a covalent Bruton’s tyrosine kinase inhibitor (BTKI), in various lymphoproliferative disorders, including CLL and MCL. In a phase 2 trial, patients affected by relapsed or refractory MCL were given daily oral ibrutinib, which led to an ORR of 68% and complete remission in 21%. A longer-term follow-up confirmed these data, with median progression-free survival of 13 months and median overall survival of 22.5 months. Thanks to these

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Figure 4. Progression-free survival (PFS) and overall survival in patients in diffuse large B-cell lymphoma patients treated with R-CHOP or lenalidomide in association with R-CHOP (R2-CHOP).

Idelalisib is an oral inhibitor of phosphoinositide 3-kinase (PI3K) that was recently published in a phase 2 trial in 50 relapsed or refractory MCL patients who received continuous idelalisib until progression or toxicity plus rituximab once a week during cycle 1, then once every cycle up to 2 years. The ORR was 88%, with complete remission in 44%. The only grade 3 adverse event, in 10% of patients, was atrial fibrillation. Recently, a multicenter, phase 3 trial that enrolled 280 patients with relapsed or refractory MCL, randomly assigned to receive daily idelalisib or weekly temsirolimus, showed that progression-free survival was significantly improved with idelalisib versus temsirolimus (hazard ratio, 0.43; median progression-free survival was 14.6 vs 6.2 months, respectively). Idelalisib was also better tolerated. All these data support the positive benefit of idelalisib in relapsed or refractory MCL, even in the blastoid variant. Ibrutinib has also been investigated in DLBCL, both as single agent in relapsed or refractory DLBCL, and in combination with R-CHOP, in first-line treatment, where it has shown restricted activity in the activated B-cell subtype. Phase 2 and 3 studies are ongoing.

- **PI3K inhibitors**
- **Idelalisib**

Idelalisib is an oral inhibitor of phosphoinositide 3-kinase (PI3K) subunits. PI3K regulates cell proliferation, survival, and motility. The α isoform plays a key role in B cells, which makes it an ideal target for therapy in lymphoma. Based on the results of a phase 1 study, an international phase 2 study with idelalisib has been conducted in 125 patients (72 with follicular lymphoma) refractory to rituximab and alkylating agents. ORR was 57% and complete remission occurred in 6%, with a median duration of response of 12.5 months. The safety profile was acceptable.

Following these results, a phase 3 trial of idelalisib plus rituximab versus placebo plus rituximab in relapsed or refractory indolent NHL was initiated. New PI3K inhibitors, targeting two different subunits, are also under investigation. Idelalisib has been studied in relapsed or refractory MCL, where it has shown less activity (with a median progression-free survival of 3.7 months).

Combination therapy with idelalisib and rituximab has been found to be synergistic. These findings are being studied in additional clinical trials in MCL.

**Conclusion**

Ongoing research in lymphomas is focusing on the role of novel agents, but many questions remain to be answered. These include identifying the most appropriate targets for novel agents and the best combinations or sequences of use of novel drugs with the aim of defining potential chemotherapy-free treatments for lymphoma patients. Prospective ongoing clinical trials in larger patient samples and longer follow-ups will add insight to the landscape of target therapy in lymphoid malignancies. For these reasons, continued participation in clinical trials should be encouraged.

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Immunotherapy, apoptosis induction, cell drug therapy, or chemotherapy: which will be the leading therapeutic axis for hematological malignancies in your country in 10 years’ time?

1. I. Aurer, *Croatia*
2. J. Briones, *Spain*
3. S. E. Goranov, *Bulgaria*
4. N. Hamerschlak, *Brazil*
5. O. Khorshid, *Egypt*
6. J. S. Kim, *South Korea*
7. I. Kryachok, *Ukraine*
8. Y.-L. Kwong, *China*
9. D. Warzocha, *Poland*
10. P. L. Zinzani, *Italy*
Treatment of cancer is fraught with difficulties resulting from limited differences between normal and malignant cells. The mainstay of systemic treatment, chemotherapy, results in significant damage to normal organs. Advances in immunology and cell biology have enabled us to attack tumor cells more precisely. Monoclonal antibodies, small molecules targeting specific intracellular processes, and broadly acting agents called immunomodulators have been added to our anticancer arsenal. So, as in warfare, the hope arises that we can win without having to resort to conventional, bloody infantry battles. But are these hopes too far flung or not? There is no uniform answer to this question. First, not all hematological malignancies are created equal and the value of non-chemotherapeutic treatment modalities will vary depending on the disease. Second, drug use is influenced not only by efficacy and toxicity, but also by affordability.

Let’s focus on efficacy and toxicity first. Monoclonal antibodies are effective in immunogenic tumors, mostly B-lymphoid malignancies, but if used as monotherapy are rarely curative. Immunomodulators have a very broad and unspecific spectrum of activity that, except in very special circumstances (eg, 5q- syndrome [myelodysplastic syndrome (MDS)]), seems best suited to amplifying the effects of other anticancer agents.

Efficacy of targeted treatments is limited by the activation of alternative cellular pathways or by mutations, eg, chronic myeloid leukemia (CML) develops resistance to tyrosine kinase inhibitors (TKI) through mutations of TKI-binding regions. Cancer cells have an unstable genome, and the more this characteristic is pronounced, the less reliable and durable the effect of targeted therapies is. Chemotherapy is toxic and has a relatively narrow therapeutic window, but is less sensitive to minor intracellular changes. I believe that in 10 years from now nonchemotherapeutic drugs will exist for the treatment of most, if not all, hematologic malignancies. Chemotherapy-free regimens of choice will consist of targeted drugs for myeloproliferative disorders and low-grade MDS (eg, TKIs for CML, JAK-2 inhibitors for myelofibrosis and polycythemia rubra vera, etc) and combinations of monoclonal antibodies, targeted drugs (B-cell receptor pathway [BCR] inhibitors, apoptosis inducers, etc), and/or immunomodulators for indolent B-cell non-Hodgkin lymphomas (NHLs). Because of the propensity of high-grade MDS and acute myeloid leukemia to acquire additional genetic mutations, best treatment results in these diseases will be obtained by combining targeted drugs (pathway inhibitors and/or apoptosis inducers) with chemotherapy. Similarly, for aggressive lymphoid neoplasms, treatments of choice will consist of combinations of monoclonal antibodies, targeted agents (eg, BCR inhibitors for B-NHLs, histone deacetylase inhibitors for T-cell NHL, or apoptosis inducers for either), and immunomodulators with chemotherapy, similar to R-CHOP (rituximab-cyclophosphamide/hydroxydaunorubicin/oncovin/prednisone) + ibrutinib or R-CHOP + lenalidomide combinations for diffuse large B-cell lymphoma. In vitro manipulation of effector cells to stimulate antitumor immunity (eg, chimeric antigen receptor T cells) will offer possibilities of curing patients for whom less aggressive treatment approaches fail.

What about affordability? If the trend of continuously increasing anticancer drug prices does not stop, in 10 years we will have two standards in Europe. In rich Western countries, hematologic neoplasia will be treated by combinations of immunotherapy and small molecules, with the addition of low to moderately intensive chemotherapy in more aggressive diseases. CHOEP-14-, eBEACOPP-, and HD-MTX-based Burkitt lymphoma regimens will be a relic of the past. In the less rich parts of the world (including Croatia), patients will still be treated with these aggressive chemotherapy regimens and less expensive newer agents. These two standards will not differ so much in efficacy as in toxicity. Unfortunately, there will also be a third standard. In poor parts of the world, patients with hematologic tumors will receive only very limited or no treatment and their outcomes will continue to be as bad as they were decades ago.
Classical chemotherapy has been, and continues to be, the mainstay of treatment for most hematological malignancies. The use of monoclonal antibodies targeting proteins expressed on tumor cells has had a critical impact on the outcome of patients with hematological malignancies, in particular those with B-cell lymphomas, and now constitutes an essential part of therapy.

In recent years, new drugs targeting different biological pathways involved in the pathogenesis of hematological malignancies have been developed and entered the clinical scene. Proteasome inhibitors, B-cell receptor signaling inhibitors (BCRIs), histone deacetylase inhibitors, and drugs targeting apoptosis-related genes are among the new classes of drugs that are changing the therapeutic landscape of our patients with hematological tumors and, more importantly, improving their survival. While in some diseases a combination of monoclonal antibodies and chemotherapy remains the best treatment, “chemotherapy-free” approaches (ie, tumor-targeting antibodies plus BCRIs or bcl-2 inhibitor combinations) are showing outstanding efficacy in some tumors. These therapies, which are clearly replacing some chemotherapy regimens in diseases such as chronic lymphocytic leukemia and multiple myeloma, now constitute first-line therapy in these indications. It seems clear that the potential therapeutic benefit of these new drugs is enormous, and it could be even greater once we learn how to better combine these drugs to maximize their antitumor effect, while reducing their toxicity.

Although the drugs described above have had an outstanding impact in the treatment of hematological malignancies, another newer class of drugs, cellular immunotherapy, is also set to markedly influence the treatment of patients with hematological cancer. The relationship between cancer and the immune system has been recognized for more than 150 years. However, it has not been until the last few years, with progress in T-cell recognition, activation, and costimulation, that physicians have begun to exploit this knowledge for cancer therapy. Two of the most exciting therapeutic developments are based on the manipulation of costimulatory pathways controlling T-cell function. The first involves the use of monoclonal antibodies targeting costimulatory or inhibitory T-cell molecules to enhance the antitumor effect of tumor-infiltrating T cells; agonistic OX40 and 4-1BB antibodies, and CTLA-4- and PD-1/PDL-1–blocking antibodies, respectively, represent clear examples of these new drugs, and more are on the way. The second involves enhancing the functional cytotoxic properties of a patient’s own T cells via genetic modification with tumor-antigen receptors and costimulatory molecules (so-called chimeric antigen receptors [CARs]). These two forms of immunotherapy were recognized as “breakthroughs of the year” by the scientific community in 2013. Preliminary results of cellular immunotherapy with CAR T cells are impressive and have revolutionized the therapeutic landscape of hematological malignancies. This new form of treatment is in its infancy, but once logistical limitations are overcome and with further refinements that improve efficacy and reduce toxicity, cell immunotherapy will take its place at the top of the list of drugs for cancer treatment. Moreover, new versions of these “cell drugs” are being developed that will certainly be more effective against cancer: tumor-infiltrating lymphocytes that recognize tumor-specific mutated peptides; and the so-called ImmTacs, a monoclonal specific T-cell receptor coupled to an anti-CD3 antibody. Importantly, these cellular drugs could be combined with antibodies targeting T-cell costimulatory or inhibitory checkpoint molecules to further enhance their efficacy. In fact, one of the actual challenges is to devise the combinations that perform best while preserving safety.

In the next decade, cellular therapies—with their different modalities—and immunotherapy with antibodies targeting costimulatory and checkpoint molecules will be at the top of the list of therapies for treating hematological malignancies. It is expected that combinations of these different modalities will give the best results. For that, well-designed clinical trials will be needed to select the best combinations to eventually replace classical chemotherapy.

**2. J. Briones, Spain**

Dr Javier BRIONES, MD, PhD
Chief of Section, Hematology Service
Associate Professor, Autonomous University of Barcelona, Hospital Santa Creu i Sant Pau
Barcelona, SPAIN
(email: jbriones@santpau.cat)

_The main therapeutic axis in hematological malignancies in 10 years’ time_
In the Republic of Bulgaria, there are approximately 8500-9000 patients with malignant blood diseases, who are treated at six university clinics plus the Military Medical Academy and National Center for Hematological Diseases, which is an advantage for a small country like ours. These centers have advanced diagnostic capabilities, imaging techniques (including PET-CT), and modern nuclear technology.

In a situation of scarce financial resources, therapeutic approaches in patients with hematological malignancies are oriented towards the most effective treatment options for young patients and the challenges of the elderly population: first-line therapy, the therapy of relapsed and refractory disease, craving for extension in PFS, and eradication of minimal residual disease. There are hematological malignancies (HMs) where there are no alternative treatments because of the frequency and quality of therapeutic response, eg, tyrosine kinase inhibitors in chronic myeloid leukemia (CML) and ATRA APO in acute promyelocytic leukemia. Most HMs, however, are treated with a combination of various methods. Modern therapeutic options comprise immunotherapy, inducers of apoptosis, cell therapy, and conventional chemo- and radiotherapy. They operate at different phases of the malignant cell cycle and predispose to enhancing therapeutic efficiency. However, in the next 10 years, I do not expect such a massive combined approach. Moreover, there are number of unspecified details in such a therapeutic strategy and more is not always better. The attempt to choose a single therapeutic method is not appropriate. I think immunochemotherapy as the primary therapeutic axis will remain the first-line treatment because of the great opportunities for new and already proven combinations. In the most common malignancies—lymphoproliferative disorders—established staging systems and prognostic indexes are applied, and data from the gene expression profile and the influence of the microenvironment are taken into account. Such risk stratification largely determines therapeutic behavior. The two most common lymphomas—diffuse large B-cell lymphoma (DLBCL) and follicular non-Hodgkin lymphoma (NHL)—have a different biology, evolution, purpose of treatment, and therapeutic response. Despite these significant differences, these are an example of modern therapeutic modeling of the main partners, immuno- (targeted therapy) + chemotherapy. Here the possibilities vary: the new generation of anti-CD20 and anti-CD30 monoclonal antibodies (ofatumumab, GA101, brentuximab vedotin) in combination with chemotherapeutic agents; increasingly widespread use of bendamustine (in indolent lymphomas); non-cardiotoxic anthracyclines, eg, pixantrone; or salvage therapy.

Such combination therapy in DLBCL results in response rates of 50%-80%. Another similar combined approach as standard of care applies to relapsed or second/consecutive treatment lines of patients with NHL: salvage regimens + autologous stem cell transplantation (ASCT). The immunochemotherapeutic effects have not been exhausted, although a considerable proportion of patients do not achieve a sustained response. In multiple myeloma, for instance, the combination of proteasome inhibitors, immunomodulators, and monoclonal antibodies ± ASCT has resulted in an unsurpassed-so-far median survival of 70 months. It should be noted, however, that therapy with 4-5 component regimens (total therapy) does not result in improved survival compared to 2-3 component regimens. Currently, there are many drugs in clinical trials burdened with great expectations, in particular agents that influence apoptosis that suppress the overexpression of the antiapoptotic proteins BCL-2, BCL-XL, or BCL-W. These will be saved for patients with unfavorable prognostic factors, individual patterns of gene expression profile, refractory disease or relapse, or elderly patients with accompanying organ dysfunction. In modern hematology, hematopoietic stem cell transplantation (HSCT) is another critical therapeutic option. In the last few years, our country has made a certain amount of progress in HSCT. To the two working transplant centers in the country in 2015, two more have been added (at the university hospitals in Plovdiv and Varna). On the one hand, this will increase transplant activity in Bulgaria and, on the other, it will also enable the development of cell therapy in mainstream transfusion, immunomodulation, and reparative medicine.

Stefan Emilov GORANOV, MD, PhD, DMsc
Professor, Head of the Clinic of Hematology and Stem Cell Transplantation, University Hospital “Sv.Georgi”, Plovdiv, BULGARIA
(email: stefangoranov@yahoo.com)
Until a few years ago, the use of cytotoxic drugs was the principal cornerstone of malignant hematologic disease therapy. Recently, extensive research has led to a change in the treatment of this group of diseases; treatment now incorporates other strategies, such as immunotherapy, apoptosis-inducing drugs, cell drug therapy, and new chemotherapy for use in refractory and relapsed diseases or first-line as a less toxic way to treat our patients. Many patients previously considered unfit for treatment can now receive effective therapy and even be cured.

However, although efficient and promising, modern therapies have also resulted in a considerable increase in health-care costs. In recent years, a series of structural changes have brought attention to Brazil, the fifth largest and fifth most populated country in the world with an estimated population of 192 million. Brazil has become an important country economically. However, particularities of the Brazilian health-care system and high costs have hampered the introduction of recent discoveries in oncology to most of the population. Just as an example, although lenalidomide and bendamustine have been approved all over the world, they have not yet been approved in Brazil. So, it is difficult to anticipate the scenario 10 years from now.

In 2015, four new agents were approved by the Food and Drug Administration (FDA) for multiple myeloma: panobinostat, ixazomib, daratumumab, and elotuzumab. Each of these takes advantage of a different mechanistic vulnerability of the myeloma cell. Another approach is to use T cells that target the B-cell maturation antigen in patients with advanced multiple myeloma (MM), which has impressive antymyeloma activity.

The management of lymphomas and chronic lymphocytic leukemia has also improved a lot. Rituximab, one of the initial “target therapy” agents in cancer care, was approved in 1997. Since then, new target therapies such as obinutuzumab, ofatumumab, and brentuximab have been developed and approved for use in Brazil.

Today new drugs based on B-cell signaling have been developed. An inhibitor of the phosphoinositide 3 kinase (PI3K) pathway, idelalisib, and a Bruton kinase inhibitor, ibrutinib could be used alone or in combinations with other drugs. Also a BCL-2 inhibitor (venetoclax/ABT-199) can be used in patients with del(17p) relapsed or refractory chronic lymphocytic leukemia.

In acute myeloid leukemia, inhibitors of the constitutively activated Fms-like tyrosine kinase 3 (FLT3) receptor have been demonstrated to play an important role in treatment. New drugs such as blinotumumab have been used in refractory or relapsed patients with acute lymphocytic leukemia (ALL) and in some B-lymphoma patients. Chimeric antigen receptor T-cell therapy is also a good approach for these diseases.

Finally, the immunologic target agents using anti-PD-1 and anti-PDL-1 drugs have been used in several hematologic diseases with success.

Not only will newly designed drugs be available for refractory or relapsed patients, but new chemotherapy agents also, such as pixantrone dimaleate for lymphomas or vincristine sulfate liposome injection for ALL. These drugs have produced exciting results in patients with these conditions.

New, interesting drugs are under development for hematologic malignancies, showing promising results with a tolerable toxicity profile. However, most of these drugs are not approved in Brazil, and the process usually takes a long time. Unfortunately, in a developing country like Brazil, the majority of patients cannot afford these drugs. An effort has to be made by doctors, patients, health authorities, and pharmaceutical companies to help Brazilian patients receive treatment with state-of-the-art regimens.

References
Tremendous effort has been made during the past few decades to cure hematological malignancies (HMs). However, when patients relapse the option of attaining a sustainable cure becomes limited, especially if it’s a 3rd or 4th relapse. Current standard of care includes chemotherapeutic agents (given at standard doses or at high doses with stem cell transplantation), molecular target treatments, and more recently immunotherapy, for which there is growing excitement.

Our aim is to cure patients with the least toxicity. In this regard, chemotherapy has reached a plateau. During the past two decades, we have seen a breakthrough with apoptosis induced by molecular target therapy, whether in chronic myeloid leukemia through BCR-ABL tyrosine kinase inhibition or in acute promyelocytic leukemia through treatment with all-transretinoic acid and arsenic trioxide. This breakthrough occurred mainly because of the nature of these diseases, which have a single identifiable translocation. Recently in chronic lymphoid leukemia, targeting the B-signaling pathway has proven effective. Otherwise, we haven’t seen sustainable cures with molecular targets in other HMs, and relapses occur due to the development of alternative pathways that overcome apoptosis. Hence, there is an unmet need for new treatment options. There is now a better understanding of how tumor cells evade recognition and destruction by the immune system. The immune system is designed to discriminate between own and tumor tissue. However, tumors use a variety of immuno-suppressive mechanisms to evade immunity. Insight into how the immune system interacts with tumors is expanding, whether through conventional monoclonal therapies like rituximab, engineered monoclonal antibodies called bispecific T-cell engagers (BiTEs), monoclonal antibodies and pharmaceutical drugs that block inhibitory T-cell pathways (ie, PD-1, CTLA-4), or adoptive cell transfer therapy with T cells engineered to express chimeric antigen receptors or T-cell receptors.

Answering the question of how some cancer cells bypass this system and discovering how to overcome their immune escape mechanism by blocking co-inhibitory molecules, which prevent T cells from destroying malignant cells, is a breakthrough. This breakthrough has gained tremendous attention as an immunotherapy melanoma, after the unprecedented, sustainable results in melanoma. These results led to the approval of some of these drugs by the Food and Drug Administration (FDA) in March 2011, and the commencement of a huge wave of trials that are currently ongoing in almost every HM. For example, a humanized PD-1–specific monoclonal antibody, nivolumab, recently demonstrated significant 90% response rates in relapsed or refractory classic Hodgkin lymphoma. As a result, nivolumab was granted FDA breakthrough therapy status for Hodgkin lymphoma treatment after failure of autologous stem cell transplantation and brentuximab therapy, offering an important treatment option in an otherwise grim situation. Similar exciting results have been seen in relapsed refractory diffuse large B-cell lymphoma and follicular lymphoma.

Because immunotherapy is clearly a new era in oncology, the right sequence of treatments, appropriate combinations, and the efficacy of these in a frontline setting still remain open to question. If the effectiveness of immunotherapy in a frontline setting is proven, this will very likely change the guidelines of HM in 10 years’ time. The financial burden of immunotherapy will definitely hinder its use in countries with a lower socioeconomic status, and this important point should be addressed.

Having different effective therapeutic options is vital. In 10 years, progress will not only have occurred in immunotherapy, but also include more precise, more effective cell drug therapy, apoptosis induction, and chemotherapeutics. In real life, in a country like Egypt with a population of over 90 million and financial constraints, the leading therapeutic axis will be a drug that produces a sustained cure, which is safe and, importantly, financially affordable.

References
The main therapeutic axis in hematological malignancies in 10 years’ time

Therapeutic results for hematological malignancies have improved with the development of combination chemotherapy and hematopoietic stem cell transplantation (HSCT). However, serious complications—such as severe infections or organ dysfunction related to conventional chemotherapy agents—are still challenging problems. As therapeutic strategies for hematological malignancies evolve, new targeted agents such as monoclonal antibodies (mAbs) for immunotherapy or novel agents that target specific cellular processes have shown more optimistic results in patients with hematological malignancies.

Since rituximab, the first anti-CD20 mAb, was approved for non-Hodgkin lymphoma in 1997, many mAbs have shown impressive efficacy in various hematological malignancies. Because newly developed mAbs conjugated with radionucleotides, cytotoxic agents, immunotoxins, or bispecific antibodies have been shown to be therapeutically effective in various hematological malignancies, future therapeutic strategies for hematological malignancies must include these mAbs.

In addition to mAbs that bind to specific tumor cell antigens, newly developed mAbs to the immune system (tumor microenvironment), such as immune checkpoint inhibitors, have been tested in various hematological malignancies. In the future, combination therapies including direct mAbs to cancer cells and mAbs to tumor microenvironment will be an important treatment modality.

Chronic myeloid leukemia (CML) is the best example of successful targeted therapy using a small molecule agent that targets specific cellular processes. Imatinib, a small molecule inhibitor of the mutant kinase and the first tyrosine kinase inhibitor (TKI) for CML, was approved for CML in 2002. Although allogeneic HSCT was essential in CML before the turn of the 21st century, TKI therapy, which is currently recommended as first-line therapy, changed treatment algorithms in CML. Other agents that target specific cellular processes—eg, proteasome inhibitors, immunomodulatory drugs, phosphoinositide 3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway inhibitors, and targeting agents for B-cell receptor signaling, such as Bruton’s tyrosine kinase inhibitor—and epigenetic-based therapies, such as hypomethylating agents or histone deacetylase inhibitors, have also shown encouraging clinical outcomes. These novel agents have been studied in monotherapy and in combination with conventional chemotherapeutic agents or mAbs and have shown impressive results in many hematological malignancies. Continued investigation with these new targeted agents will further improve the treatment outcomes of hematological malignancies.

Although the arrival of new agents that target specific cellular processes has heralded a remarkable period of progress in the treatment of hematological malignancies, the main limitation of these therapies is loss of antitumor efficacy. This limitation is mainly related to the clonal evolution of malignant cells. As malignant cells are continuously reshaped, it is crucial to understand the exact pathways for disease progression and find the right target. Therefore, personalized precision medicine using high-throughput genomic platforms, such as next-generation sequencing, would be an area of great interest for future study.

Allogeneic HSCT is already integrated in the therapeutic strategy of many hematological malignancies. However, allogeneic HSCT is still severely hampered by its high treatment-related morbidity and mortality. More specific and less toxic cellular therapies, such as T cells or natural killer (NK) cells, have been intensively studied. Chimeric antigen receptor (CAR) enhanced T cells are T lymphocytes engineered to specifically bind to tumor cells. CAR T cells can enhance selective cancer-killing ability and act like a "living drug." Early results from CAR T-cell studies have shown impressive results in patients with hematological malignancies. Because NK cell therapy has also been emerging as a promising immunotherapy for cancer in general, many studies using NK cell or NK cell–targeted therapies are being conducted in hematological malignancies.

In the future, combination or sequential treatment using newly developed targeted agents, eg, immunotherapy or small molecule inhibitors, and personalized cell therapy based on a tumor’s specific molecular characteristics will be leading therapeutic axes for hematological malignancies.

6. J. S. Kim, South Korea

Jin Seok Kim, MD, PhD
Division of Hematology, Department of Internal Medicine, Yonsei University College of Medicine, Severance Hospital, Seoul, SOUTH KOREA (email: hemakim@yuhs.ac)
7. I. Kryachok, Ukraine

Angiogenesis plays a crucial role in the development of malignant tumors. Various research has shown that high concentrations of vascular endothelial growth factor (VEGF) are present in the tissues of patients with non-Hodgkin lymphomas and that VEGF levels in patients’ blood is associated with a negative prognosis. These findings led to anti-VEGF research in lymphoproliferative diseases.

The first registered proteasome inhibitor, bortezomib, has proven to be effective for treating resistant mantle cell lymphomas. At present, a large number of clinical phase 2 and 3 studies are being conducted to evaluate bortezomib in combination with chemotherapy or targeted medicines for the treatment of various non-Hodgkin lymphomas. Other new treatment approaches are also being developed. An inhibitor of histone deacetylase, vorinostat, has proven to be quite effective for resistant T-cell lymphomas of the skin.

The novel aza-anthracenedenedeo picantrone has been approved in Europe for the management of multiple relapsed or refractory aggressive B-cell non-Hodgkin lymphoma. The study of the effectiveness of immunomodulators in combination with other therapeutic agents for the therapy of multiple myeloma, CLL, and NHL looks quite promising.

The majority of the medicines approved for use in Europe are accessible in Ukraine. However, a considerable barrier to the widespread use of high-technology medicines is their high cost. For this reason, in Ukraine a promising approach would be to use biosimilars with confirmed efficacy and a more accessible price as well as chemotherapy drugs and their combinations, which are easy to access, for widespread use.

References

Another promising approach is the use of monoclonal antibodies in monotherapy or in combination with a course of standard chemotherapy. This increases the percentage of patients who are entirely cured without increasing the toxicity of therapy. All the antibodies, to a variable extent, possess several mechanisms of action, and the contribution of each of these is not yet clear.

Ibrutinib is one of a new class of drugs for B-cell tumors that blocks signal transmission from the B-cell receptor. Recently, it was shown the use of blockers of this receptor (ibrutinib, idelalisib) led to the achievement of remission and efficiently prolonged the life of resistant patients with chronic lymphocytic leukemia and other lymphoproliferative diseases. These medicines are effective even if there is a deletion in the 17th chromosome. Previously, only an allogenic bone marrow transplantation was an option for such patients. The principal features of ibrutinib are minimal toxicity and a convenient oral route of administration.

F or the last 10 years, hematology has been experiencing real progress in the development of new effective methods of treatment. Hemoblastosis, which used to be fast progressing and absolutely terminal in the past, is now turning into a controllable disease. What is more, some diseases can be completely cured nowadays.

Targeted therapy is a treatment that exerts influence upon a specific molecular target selectively presented in the tumor cell. Such targets can be superficial molecules—e.g., the receptors of various signaling pathways, some structural constituent parts of a cell, or intracellular proteins—the mediators of signal transmission, RNA molecules, or structural units of genes. Targeted therapy is a considerable breakthrough in the treatment of lymphoproliferative diseases. Rituximab, ibritu- monab, tositumomab, denileukin diftitox, bortezomib, thalidomide, lenalidomide, and temsirolimus are today widely used antineoplastic drugs for the therapy of different types of lymphoma. The use of cellular technologies, monoclonal antibodies, and BCL-2 inhibitors seems promising. Targeted therapy cannot yet replace standard chemotherapy, but it does allow us to increase the essential effectiveness of therapy, using a combined approach. 

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The main therapeutic axis in hematological malignancies in 10 years’ time.

References
The treatment of hematological malignancies is advancing at an unprecedented pace. This advance has been fueled and driven by novel observations in molecular genetics and cellular signaling. Targeted therapy is now an inseparable component of treatment for hematological malignancies.

Chemotherapy is, in most instances, not pathology-specific. However, it continues to be a useful treatment, and a significant proportion of patients with lymphomas and, to a certain extent, leukemias can be cured with conventional chemotherapy. Moreover, it is a strategy that can be adopted at the community level.

Novel therapies are target-specific. The challenges of adopting a target-specific approach are protean. Firstly, these strategies are predicated on the premise that therapeutic targets, which may be biologic or molecular, can be identified accurately in routine practice and in a timeframe that is clinically relevant. Such a premise is only realistic in large academic centers, where state-of-the-art investigative tools are available; this may not be possible at a community level.

Secondly, physicians must be well-trained to be able to keep abreast of therapeutic discoveries, in order to utilize these advances for the benefit of their patients. Shifts of paradigm occur with such regularity that continuous medical education is now an important issue that medical communities have to contend with.

Thirdly, targeted therapies are expensive. Affordability becomes a pivotal problem intricately linked to reimbursement policies, which are in turn determined by health-care investment and the economies of different countries.

Finally, every population is aging. The goals of treatment may not necessarily be curative, in the conventional sense, for elderly patients, who can often live a reasonable life with detectable disease. The implication is that there will be a demand for medications that will maintain good control of disease without its eradication, and many targeted drugs fall into this therapeutic category.

In developing economies, chemotherapy is likely to still remain the mainstay of treatment of hematological malignancies for the next decade. The ability to confirm the presence of the requisite therapeutic targets is not routinely available, and physicians may not be trained to handle the toxicity profile of these newer medications. Furthermore, cost is a major concern. However, in tertiary and quaternary centers, targeted therapy will increasingly be used. Current strategies combine immunotherapy (naked or conjugated monoclonal antibodies) with drugs that affect cellular signaling. In B-cell malignancies, for instance, a monoclonal antibody against CD20 is often combined with a signal transduction inhibitor. Induction of apoptosis is another strategy, and specific points in the apoptosis pathway may be targeted. As the efficacy apparently varies depending on the malignancies and their dependence on derangement of apoptosis as the major survival pathway, a combination of an apoptosis inducer and a monoclonal antibody is again utilized. Immune checkpoint blockade is a promising novel approach, and various strategies have shown remarkable efficacy in malignancies that are refractory to numerous lines of treatment.

Therefore, in the next decade, conventional chemotherapy is still likely to be used extensively at regional and community hospitals for the treatment of hematological malignancies. Patients for whom conventional treatment fails may then be referred to tertiary and quaternary centers, where a combination of different approaches—including immunotherapy with signal transduction inhibitors, immunotherapy with apoptosis inducers, or immune checkpoint inhibition—will then be used. It will be interesting to find out if the usage of high-dose chemotherapy and hematopoietic stem cell transplantation decreases, with the expenditure of this strategy being redistributed to targeted therapy, which represents a more rational utilization of health-care resources.
The further backward you can look, the further forward you can see.” This quote from Sir Winston Churchill is the most appropriate starting point to think through and answer the controversial question given in the title. Certainly, changing paradigms in the treatment of hematological malignancies will not be an abrupt and sudden event, but rather a continuum, a process anchored in the past and based on research results. Living in the “present” of a middle-income country also gives an additional perspective to the possible magnitude of change likely to happen within a decade. Insurance reimbursement limits are however major restraints to such paradigm shifts in patient care.

The current standard for initial treatment in most hematopoietic and lymphoid tumors is still chemotherapy and/or immunochemotherapy. Thus, most likely, this will remain a solid foundation for future adjustments. The insights into the genetic basis of these tumors gained from next-generation sequencing studies indicate that most tumors possess multiple structural abnormalities that result in activation of multiple pathways. One can thereby expect that upcoming clinical studies will focus on rational combinations of targeted drugs (signal transduction modifiers, kinase inhibitors, “epigenetic” drugs, and apoptosis modulators) or combinations thereof with conventional therapeutics.

Recently published studies indicate that immunotherapy has emerged as an extremely promising approach to treat hematological malignancies. The concept of using the body’s own immunity against cancer is fascinating, and holds promise for completely eradicating tumor cells. Indeed, after publication of very impressive papers highlighting the clinical efficacy of checkpoint inhibitors, immunotherapy research has burgeoned forth in recent years and will bring clinically applicable results a few years on from now. There are multiple immunotherapeutic strategies that have currently achieved clinical approval or are in the phase of clinical testing.

Monoclonal antibody–based immunomodulation is the most likely strategy to succeed for standard practice in a clinical setting, due to its simplicity, safety, and moderate costs. We can expect this strategy to be commonly adopted in clinics in the upcoming decade. More advanced therapies—including cell-based therapies (eg, chimeric antigen receptor T cells or personalized vaccines)—require a more sophisticated environment and more specialized infrastructure, and are associated with much higher costs. Development of these approaches will continue, but such therapies are likely to be available only in highly specialized centers.

Given the approaching era of immunotherapies, a middle-income country faces a huge challenge, but also an opportunity, to be among the leaders of this change; if not accepted, we will only be the end users of technologies developed elsewhere.

Developing innovative therapies/immunotherapies and advancing the rating of the Polish R&D sector requires coordinated support and increased research expenditures (Poland falls below the EU average, with less than 1% of gross domestic product spent on research). Although the importance of support and innovation in research has recently been much highlighted in Poland, there is still a gap in friendly and fruitful collaboration between Polish and European/American research institutions. To continue the transformation of Polish science and increase the chances of our patients receiving innovative therapies, researchers and authorities should work together on closing such gaps in the upcoming decade.
Dramatic progress in the understanding of underlying disease biology and the development of novel therapeutics has yielded a revolution that is poised to transform the face of lymphoma treatment across a broad spectrum of histologies. Ongoing strategies to improve the success of drug development include the use of biomarkers to select patients for specific therapy and the development of mechanism-based combination regimens.

In fact, despite the entrenched role of chemoimmunotherapy as the initial treatment of choice for high-tumor-burden follicular lymphoma and mantle cell lymphoma patients, rituximab-lenalidomide, often called “R2”, is emerging as a potent chemotherapy-sparing competitor.1

In the past few years, several drugs have obtained regulatory approval for the treatment of lymphoma, including the antibody drug conjugate brentuximab vedotin, a novel glycol-enginered anti-CD20 antibody obinutuzumab, the B-cell receptor signaling inhibitor brutinib,2,3 the PI3K-delta inhibitor idelalisib,4 and the immune modulatory drug lenalidomide.

There are also multiple as-yet-unapproved targeted drugs with promising efficacy, including the Bcl-2 inhibitor venetoclax,5 a second-generation inhibitor of Bruton’s tyrosine kinase (acalabrutinib), and several antibody-drug conjugates. In addition, immune therapies have exciting potential, including monospecific and bispecific antibodies, immune checkpoint inhibitors,6 and engineered chimeric antigen receptor T cells. Therefore, the landscape of new drugs for lymphoma has become crowded, necessitating rational prioritization for development and selection of combination therapies.

References

With more than 600 drugs in clinical or preclinical development for the treatment of cancer, the number of possible combinations is overwhelming. Therefore, a prioritization plan for the development of novel combinations should be mechanism-based, rather than empiric. Furthermore, it is important to identify biomarkers to select patients whose tumors harbor the relevant pathways for the targeted therapy.

The convenience of oral drugs for cancer therapy is associated with new challenges, including the benefit of prolonged administration, compliance, cost, and late toxicity. The optimal duration of therapy in patients achieving complete remission should be carefully addressed, in addition to the role of retreatment strategies.

Finally, in this era of effective and tolerable chronic administration of novel agents, we are witnessing improved progression-free survival even in patients whose treatment response may not meet the definition of response. Accordingly, patients with “minor” tumor reduction are also benefiting from modern therapy. These observations mandate that the current and traditional response criteria be revised to reflect patient benefit, rather than depend on arbitrary cutoffs, to define treatment response. Furthermore, as some new trials are selecting patients based on genetic biomarkers and not tumor types, an accurate assessment of efficacy across tumor types will benefit from common “response criteria” for lymphoid tumors.

The main therapeutic axis in hematological malignancies in 10 years’ time

P. L. Zinzani, Italy

Pier Luigi ZINZANI, MD, PhD
Professor of Hematology, Institute of Hematology “Seràgnoli”, University of Bologna, Bologna, ITALY
(email: pierluigi.zinzani@unibo.it)
Leukemias, lymphomas (Hodgkin and non-Hodgkin [NHL]), and myelomas are associated with increasing age and so their prevalence is likely to rise because of population aging worldwide. Conventional treatments—chemotherapy, surgery, and radiation therapy—suffer from low specificity and high toxicity, and to overcome these barriers Servier is concentrating its R&D on inducers of apoptosis, tumor-targeting antibodies, and immunotherapy.

The first inducer of apoptosis from the Servier pipeline is Pixuvri® (pixantrone dimaleate), which is indicated for multiply relapsed aggressive B-cell NHL and is currently in phase 3 studies of NHL, in combination with the monoclonal antibody rituximab. Another inducer of apoptosis, a Bcl-2 inhibitor, has potential indications in NHL, chronic lymphocytic leukemia, and acute myeloid leukemia (AML), while an Mcl-1 inhibitor is in preclinical development to target AML, lymphoma, and multiple myeloma. In partnership with MacroGenics, Servier is also developing tumor-targeting antibodies using dual-affinity retargeting (DART®) technology. Phase 1 studies are currently testing a DART molecule in AML and myelodysplastic syndromes. Lastly, in tandem with Cellectis, Servier is developing UCART19, an allogeneic T-cell product for treatment of CD19-expressing hematological malignancies. Servier has recently initiated two clinical studies with UCART19 in pediatric and adult leukemias.

Hematological malignancies: the situation today

Leukemia, in all its forms (notably acute and chronic), affects some 9000 people every year in France, and in the United States about 60 000 new cases are expected in 2016. A cancer of the bone marrow and blood, leukemia is classified into four main forms according to cell type and rate of growth: acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), and
chronic myeloid leukemia (CML). The risk of leukemia is increased in people who have undergone chemotherapy and in children with Down syndrome and some other genetic abnormalities, but those at risk have been exposed to ionizing radiation. A famous instance is that of two-time Nobel Laureate Marie Curie, whose research work resulted in long-term exposure to radioactivity, the dangers of which were unrecognized at the time. She died of leukemia, as did her scientist daughter Irène, whose discovery with her husband, Frédéric Joliot-Curie, of artificial radioactivity won them the Nobel Prize in Chemistry in 1935.

Although often seen as a pediatric cancer (it accounts for one-third of childhood cancers), leukemia is usually diagnosed in adults, in whom the probability of it developing increases with age, particularly among the over-70s. The most common types among adults are CLL and AML, while ALL accounts for 75% of cases among children and teenagers. Incidences of the different types of leukemia have been rising in recent decades, but death rates have declined and advances in treatment have increased five-year survival rates.1

Usually grouped with leukemias are MDS, which are sometimes termed preleukemia anemias. MDS, known also as bone marrow failure disorders, are cancers in which blood cells in the bone marrow fail to mature. Mainly a disease of the over-60s, MDS are more common among white males. Repeated exposure to benzene resulting from smoking tobacco or exposure to secondhand cigarette smoke is a possible cause of MDS, and of AML.²

Lymphomas are a heterogeneous group of neoplasms once believed to be clearly distinct from leukemias, but in fact the differences are often vague. The two main types are Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). In the USA, almost 600 000 people (182 000 HL, 607 000 NHL) are estimated to be currently living with, or in remission from, lymphoma. Estimates from the American Cancer Society suggest that, in 2016, about 81 000 new cases will be diagnosed and some 21 000 people will die from lymphoma, mostly NHL, the seventh most common cancer in the USA.¹

As with most cancers, the risk of developing NHL increases with age. In contrast, the risk of HL increases during adolescence and early adulthood, before decreasing during middle age and rising again in later life. Identified risk factors are mostly associated with altered immune function. Survival varies widely by subtype and disease stage, but overall 5- and 10-year relative survival rates are quite high (in the USA, respectively 70% and 60% for NHL, and 86% and 80% for HL).³

Myeloma, a malignancy of plasma cells, can occur in the ribcage, pelvis, and spine, and wherever there is bone marrow, which produces these and other blood cells (red cells, platelets, white cells, and so forth). This multiplicity of sites explains why this hematological cancer is sometimes called multiple myeloma. Part of the immune system, plasma cells secrete antibodies in response to infections. When they become malignant, however, the resulting myeloma cells produce abnormal antibodies, which are deposited in various organs, resulting in anemia, bone pain, renal failure, polyneuropathy, and other symptoms.³,³

Rarely diagnosed in people under 40 years of age (median age at diagnosis is 69), myeloma is expected to be diagnosed in around 30 000 people in the USA in 2016, where its incidence has been rising, though mortality has decreased slightly in recent times.¹

Conventional treatments of blood cancers
Chemotherapy is the gold standard in the management of most types of leukemia, and of MDS (possibly with radiation and stem cell transplantation), and uses various anticancer drugs alone or in combination. As for lymphomas, NHL and HL are also mainly treated by chemotherapy, sometimes along with radiation therapy. If these prove ineffective, stem cell transplantation is an option, as is the use of a monoclonal antibody linked to a chemotherapeutic drug. Myeloma is treated principally using chemotherapy, steroids, and targeted (biological) therapies (eg, thalidomide, bortezomib, lenalidomide), so-called because they target proteins on the surface of or within cancer cells. Such treatments are used to halt disease progression, control symptoms, and improve quality of life because, although treatable, myeloma is seldom curable.

Despite these standard treatments and substantial therapeutic advances in the management of hematological malignancies, unmet needs remain and Servier has set itself the task of addressing them. Management of AML, for example, is in great need of more effective targeted therapies, and in hematological malignancies in general there is a need to reduce rates of relapse and to increase survival rates in patients who experience relapse. Treatment of hematological malignancies has evolved substantially in recent years, notably through the emergence of targeted therapies, whose development is underpinned by our increasing grasp of disease pathogenesis.
Analysis by Global Business Intelligence shows that the blood cancer pipeline is an exemplar of innovation in the healthcare industry and prioritizes targeted therapies, as opposed to chemotherapy. In a June 2016 report, Global Business Intelligence noted that worldwide there are currently 1234 pipeline drugs under development for hematological malignancies: 798 for leukemia, 552 for lymphoma, and 396 for myeloma (note that some drugs are intended for more than one disease).

Servier and hematological malignancies: a triptych of technologies
Servier is committed to becoming a leading player in the field of hematological malignancies. Only innovative treatments underpinned by robust understanding of carcinogenesis will bring relief to the millions of people around the world who are living with blood cancers. To this end, Servier is following three main lines of research: inducers of apoptosis, tumor-targeting antibodies, and chimeric antigen receptor (CAR) T cells.

◆ 1. Inducers of apoptosis
Apoptosis, otherwise known as programmed cell death, is a genetically controlled process that plays a key part in tissue development, homeostasis, and disease, notably malignancies. For instance, if cells evade apoptosis, they may proliferate anarchically, leading to cancer. Evasion of apoptosis is also a common mechanism of resistance to chemotherapeutic drug treatment.

◆ Bcl-2 inhibitors
Bcl-2 is one of a family of regulatory proteins that promote cell survival by sequestering and inhibiting two proteins, Bax and Bad, which normally trigger cell death. Inhibitor of Bcl-2 proteins would, therefore, be expected to restore the apoptotic capacity of cancer cells. However, apoptosis is a natural physiological process and it is hard to design molecules specific for Bax and Bad that do not also affect other pathways: previous research has yielded inhibitors that block several Bcl-2 proteins, resulting in too many side effects (notably, platelet toxicity) for clinical use.

Servier and Vernalis have been working in partnership since 2007 to discover specific inhibitors of the main members of the Bcl-2 family of proteins. This collaboration has yielded S55746, which is now in phase 1 clinical development. A pro-apoptotic agent, S55746 is a selective antagonist of Bcl-2 with indications in NHL, CLL, AML, MDS, and myeloma, and a good safety profile. Servier and Novartis are cooperating together on the development and commercialization of S55746.

◆ Mcl-1 inhibitors
Servier, together with Vernalis, is currently working on the preclinical development of other inhibitors of the prosurvival members of the Bcl-2 family of proteins, notably an inhibitor of Mcl-1 (induced myeloid leukemia cell differentiation protein).

Preclinical data show that Mcl-1 inhibitors, alone or in combination with targeted therapies, are highly active against models of hematological malignancies. Potential indications include myeloma, AML, and lymphoma.

◆ Other drugs that induce apoptosis
First developed by CTI BioPharma, with whom Servier has been in close partnership since 2014, pixantrone (Pixuvri®) was initially viewed as an anthracycline with the advantage of decreased cardiotoxicity. We have since discovered, however, that pixantrone is a weak topoisomerase II inhibitor. It forms stable DNA adducts, thus preventing DNA replication and transcription, resulting in progressive loss of chromosomes, mitotic catastrophe, and cancer cell death after several divisions.

Phase 3 results of pixantrone were published in 2012. In a multicenter, open-label, randomized trial, 140 patients with aggressive NHL were randomly allocated to receive either up to six cycles of pixantrone dimaleate (85 mg/m² intravenously on days 1, 8, and 15 of a 28-day cycle; n=70) or the physician’s choice of treatment (vinorelbine, oxaliplatin, ifosfamide, etoposide, mitoxantrone, or gemcitabine; n=70). At the end of the treatment period, in pixantrone-treated patients the response rate was significantly higher and progression-free survival was longer. Even better, these results were achieved without major side effects, all toxicities being manageable. Pixantrone has a good safety profile because it results in limited production of reactive oxygen species and it has less potential to accumulate in cardiac tissue.

Pixantrone has conditional marketing approval in Europe as a monotherapy in adult patients with multiple relapsed or refractory aggressive NHL. An ongoing phase 3 randomized, multicenter trial is assessing the efficacy of pixantrone plus rituximab versus gemcitabine plus rituximab in patients with diffuse large B-cell lymphoma or follicular grade 3 lymphoma. Further studies are needed to establish whether pixantrone can be used as second-line therapy or in combination with targeted therapies.

Servier has invested much effort in the study and development of inducers of apoptosis and we have high hopes for the development of other proapoptotic molecules, including some first-in-class agents.

◆ 2. Tumor-targeting antibodies
Servier is developing a number of engineered antibodies in partnership with the biopharmaceutical company MacroGenics. Using dual-affinity re-targeting (DART®) technology, we are able to create antibodies, antibody derivatives, and antibody-like molecules for use as therapeutic agents. These DART molecules are bispecific antibody-like proteins that target two different antigens. For instance, one such DART molecule, called DART CD3/CD123 (S80880), which we are de-
blood and genetically engineered to produce proteins called chimeric antigen receptors (CARs) on their surface. CARs enable T cells to recognize a specific antigen on cancer cells. The CAR T cells are grown in the lab and infused into the patient’s body, where they multiply, recognize, and kill cancer cells bearing the specific antigen.12,13

This process must be repeated for each patient, which means it is a truly personalized treatment, the downside being that it is time-consuming and labor-intensive. Servier and Cellectis have therefore developed allogeneic CAR T-cell therapy, for which Servier has an exclusive licensing option, in which T cells from healthy donors, rather than from the patient, are used. A single preparation of UCART (universal chimeric antigen receptor T [cells]) could therefore be used for several, possibly many, patients. These UCART19 cells (S68587) target CD19, an antigen expressed by CLL and ALL cells. The attendant risk of using allogeneic CAR T cells, rather than autologous T cells, is that they could induce graft-versus-host disease. We have circumvented this problem by using Cellectis proprietary gene editing technology to inactivate the endogenous T-cell receptor gene expressed by the starting T cells. Significant manufacturing and clinical challenges lie ahead, but the hope is that UCART19 will become an off-the-shelf therapy for patients with hematological malignancies.

UCART19, which is currently in preclinical development for use in CLL and in phase 1 studies of ALL, has also been used in a “last chance” compassionate care protocol. In June 2015 at Great Ormond Street Hospital in London, UCART19 cells were successfully used to treat a baby girl in whom all attempts to treat refractory, relapsed ALL had failed. Given just months to live, one-year-old Layla was given UCART19 therapy on a compassionate basis, in line with UK special therapy regulations.14 Professor Waseem Qasim, Consultant Immunologist

Figure 1. Cellular expression of CD123.
Abbreviations: CLP, common lymphoid progenitor; CMP, common myeloid progenitor; HSC, hematopoietic stem cell; LSC, leukemic stem cell; pDC, plasmacytoid dendritic cell. © 2016, MacroGenics, Inc. With kind permission.

Figure 2. The mechanism of action of DARTs in oncology.
Abbreviations: CSC, cytokine-secreting cell; CTL, cytotoxic T lymphocyte; DART, dual-affinity re-targeting; NKT cell, natural killer T cell; TCR, T-cell receptor; V_H, variable region of the heavy chain; V_L, variable region of the light chain. © 2016, MacroGenics, Inc. With kind permission.
at Great Ormond Street Hospital, was quoted as saying that: “The successful treatment of a patient with UCART19 cells represents a landmark in the use of new gene engineering technology. If replicated in other patients, it could represent a huge step forward in treating leukemia and other cancers.”

It was announced in May 2016 that a second baby with ALL at Great Ormond Street Hospital was in remission following treatment with UCART19 cells. At the time of writing, both children are doing well, but longer follow-up is needed to establish whether UCART19 therapy has cured the ALL, or simply delayed its progression. Servier has already started two clinical studies of UCART19 in pediatric and adult ALL.

**Conclusion**

Over the last 15 years or so, the development of targeted therapies has begun to revolutionize the treatment of hematological malignancies, which have more active pipeline products than respiratory and cardiovascular therapies. Servier has developed scientific collaborations with biotechnology and pharmaceutical companies with proven track records in oncology, as well as with prestigious research institutions such as the Institut Curie and the Institut Gustave Roussy. Through these partnerships and its own research, Servier is moving innovations from bench to bedside in the management of hematological malignancies.

**References**


**Keywords:** apoptosis inducer; tumor-targeting antibody; chimeric antigen receptor T cell; dual-affinity re-targeting; universal chimeric antigen receptor T cell; partnership; Cellectis; CTI BioPharma; MacroGenics; Novartis; Servier; Vernalis

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Multiple myeloma (MM) is the second most prevalent hematological malignancy and causes approximately 20% of all deaths due to hematological malignancies. Despite the significant efficacy of novel therapies at eradicating MM in vitro and in animal models, many MM patients fail to respond to therapy, especially in a relapsed setting. The discrepancy between the efficacy of laboratory models and clinical efficacy may be related to: (a) use of cultures of cancer cell lines alone, neglecting the vital role of the bone marrow microenvironment; (b) failure to recapitulate the three-dimensional (3-D) structure of tissue, with oxygen and drug gradients varying with the depth of tissue; and (c) reliance on a limited number of cell lines that fail to reflect the heterogeneity between patients, in classic tissue culture models.

We have developed a novel patient-derived 3-D tissue-engineered bone marrow (3DTEBM) model. The 3DTEBM was shown to demonstrate the interactions of MM cells with their malignant microenvironment, simulate 3-D aspects of the bone marrow niche, and reflect individual heterogeneity in progression and response to therapy in MM patients. 3DTEBM technology provides an improved model for understanding tumor biology and drug resistance of MM in the context of the malignant bone marrow microenvironment, as well as provides personalized prediction of therapeutic efficacy in individual MM patients. Current studies are being conducted to expand the use of 3DTEBM to other hematologic malignancies and solid tumors.

Multiple myeloma (MM), the second most prevalent hematological malignancy, causes approximately 20% of all deaths due to hematological malignancies. The American Cancer Society estimates that in 2016 about 30,000 new cases will be diagnosed and about 12,500 deaths will occur. Newly diagnosed MM patients with standard-risk disease have a median overall survival of about 4 years, whereas high-risk disease MM patients have a median overall survival of about 2 years.1

Recent advances in understanding of the molecular mechanisms of malignancy in MM cells, as well as their interaction with the bone marrow microenvironment, have led to the development of novel therapies.2,3 Over the past decade, MM patients’ survival and response to therapy has improved significantly due to the introduction of these novel therapies.4 Such therapies include: immunomodulatory drugs (thalidomide, lenalidomide, and pomalidomide), proteasome inhibitors (bortezomib, carfilzomib, and ixazomib), antiangiogenic and immunomodulatory therapies, and plasma cell-directed antibodies.
marizomib, and ixazomib), monoclonal antibodies (mAbs) (elo-
tuzumab, siltuximab, and daratumumab),2 as well as molec-
ular targeted therapies for the treatment of relapsed/refrac-
amentary MM, including cell signaling targeted therapies (HDAC,
PI3K/AKT/mTOR, p38 MAPK, Hesp90, Wnt, Notch, Hedge-
hog, and cell cycle) and strategies targeting the tumor micro-
environment (hypoxia, angiogenesis, integrins, CD44, CXCR4,
and selectins).3 However, despite the significant efficacy of
these therapies at eradicating MM in vitro and in animal mod-
els,2 40% of upfront patients and more than 90% of relapsed
patients fail to respond to therapy.2,3 The discrepancy between
clinical outcome and laboratory models points to fundamen-
tal problems in our biological models for drug development in
cancer, and particularly in MM.

The discrepancy between laboratory and
clinical outcomes
The discrepancy between drug efficacy in laboratory models
and the shortcomings in clinical efficacy may be related to
several limitations of the current models used for studying
tumor biology and for testing drug efficacy, especially in the
in vitro setting.

A. The role of bone marrow microenvironment
in drug resistance
Most in vitro models use cultures of cancer cell lines alone
and neglect the vital role of the bone marrow microenviron-
ment in tumor progression and drug resistance, which has
been shown to play a critical role in the resistance of MM cells
to therapy.5,11 Components of the bone marrow microenvi-
ronment include: stromal cells, osteoclasts, osteoblasts, im-
mune cells, endothelial cells, and extracellular matrix (ECM).
Each component interacts with tumor and other cells, pro-
moting drug resistance (Figure 1A).

Therapy resistance acquired by MM cells is one of the main
problems in MM treatment. The bone marrow microenviron-
ment plays a crucial role in the development of drug resistance
in MM; direct and indirect interaction of MM cells with the
bone marrow microenvironment induces drug resistance in these
cells.5-8 Disruption of the interaction between MM cells and
the bone marrow microenvironment has been introduced as
a novel strategy for sensitization of MM to therapy.6 Drug resist-
ance may, moreover, be induced by intrinsic mechanisms of
cancer cells (genetic alterations and signaling pathways). Ge-
netic alterations (including translocations, deletions, and am-
plifications) have been shown to upregulate oncogenes and
downregulate tumor-suppressors.12 In addition, deregulated
cell signaling pathways, including the p53-mediated apoptosis
pathway,13 NF-κB pathway,14 endoplasmic reticulum stress,15
Wnt signaling pathway,16 and hypoxia-inducible factor (HIF)
signaling pathway,17 are a key factor of drug resistance in MM
cells. Drug resistance in MM may also develop due to drug
efflux by transporters, such as P-glycoprotein.18 However, the
mechanisms leading to the deregulation of these pathways
in MM is not fully understood, and deeper elucidation of these
mechanisms will improve our knowledge of the pathophys-
iology of MM and development of new therapeutic targets to
overcome drug resistance.

B. 3-D aspects of the bone marrow niche
Tumor tissue is a 3-D structure with both oxygen and drug
concentration gradients that vary with the depth of tissue.19
Hypoxia (low oxygen) has been shown to increase drug resist-
ance in MM and other cancers.20,21 Drug diffusion is also lim-
ited by the depth of tissue, which restricts the efficacy of treat-
ment (Figure 1B).
Tumor hypoxia is a phenomenon that has been known about for at least 60 years. It is observed in growing malignant tumors and leads to therapy resistance. Hypoxia, which develops due to uncontrollable tumor cell proliferation, correlates with poor prognosis in cancer patients. Hypoxia confers treatment resistance to cancer cells by regulating processes such as: (i) cell cycle arrest (quiescence), a state of reduced cell proliferation which protects the cells from external stress; (ii) inhibiting apoptosis and senescence of cells; and (iii) controlling autophagy, endoplasmic reticulum stress, and p53 and mitochondrial activity. Hypoxia induces dedifferentiated and immature cell phenotypes (as shown in neuroblastoma and breast cancer and in MM), as well as maintaining stem cell phenotype in both hematopoietic stem cells and in brain stem cells. This was confirmed by activation of stemness-related genes and pathways, such as Oct4, HIF, Notch, Wnt, and Hedgehog. Hypoxia induces clonal selection and genome changes by inducing the stemness of the cell fraction residing in the most hypoxic region. Hypoxia modulates gene expression mainly via HIF-1 and HIF-2, potent transcription factors that upregulate target genes, leading to hypoxia tolerance that enables survival and proliferation, and determines tumor sensitivity to treatment. HIF-1 thus represents a therapeutic target, the manipulation of which may re-sensitize drug-resistant cells. Similarly, inhibition of HIF-2 has been shown to reverse resistance to doxorubicin and etoposide.

However, classic two-dimensional (2-D) in vitro tissue culture systems do not recapitulate 3-D oxygen gradients and thus cannot portray a phenomenon like hypoxia. There is an urgent need to develop a model that reflects 3-D aspects of the bone marrow niche, including hypoxia. A good model should mimic all biological roles in tumor biology, including drug gradients. However, currently used tissue culture models generally rely on a limited number of cell lines, which limits their ability to mimic the enormous heterogeneity between individual patients. Thus, a new model that takes into consideration personal heterogeneity between patients by deriving an autologous model from individual patients, would be welcome.

### Problems in current models used for studying multiple myeloma

**In vitro 2-D cultures**

Classic 2-D culture models of MM are widely used in an experimental in vitro setting. However these models mostly utilize MM cell lines alone, which clearly fail to reproduce the effects of the bone marrow microenvironment, 3-D aspects of bone marrow (such as drug and oxygen gradients), and personal heterogeneity between patients. In some cases MM cells are used in coculture with other accessory cells, including stromal cells, endothelial cells, and/or the ECM. Although this all adds value to understanding the interaction of MM cells with the bone marrow and its role in drug resistance, it still lacks the 3-D and personal heterogeneity aspects of the disease (Figure 2A).

**In vitro 3-D models**

To overcome some of the limitations of the 2-D models, polymer-based in vitro 3-D models were developed for MM. The models were based on acrylic polymers, Matrigel scaffolds, and silk fibers. While these 3-D models provide better alternatives to 2-D cultures, these systems are based on synthetico materials that are not naturally found in bone marrow, which may cause significant changes in the interaction between the different components of the culture. Moreover, none of these models were shown to support proliferation of primary MM cells and they rely mainly on MM cell lines, which limits their ability to reflect individual heterogeneity in the patient population (Figure 2B).

**In vivo models based on bone chips**

Fetal bone chips are implanted in immune-deficient SCID (severe combined immunodeficiency) mice. After the fetal bones grow in the mice (over 6-8 weeks), MM cells are injected into the bone to allow progression of MM in the human bone chips in the mice. This model resembles bone marrow physiological conditions more closely; however, it relies on a normal bone marrow microenvironment, which has been shown to have different effects on MM cell growth compared to the malignant microenvironment of MM. In addition, this model is costly and labor intensive; it requires 6 weeks for the growth...
of the bone in mice and 4 more weeks for the growth of the MM cells in the bone (10 weeks in total), which adds more challenges to the technical feasibility of the model.

**In vivo xenograft models**

MM cell lines that are implanted directly into immune-deficient SCID mice are widely used. Subcutaneous injection of the cells is common, but this lacks all aspects of the interaction between MM cells and the bone marrow microenvironment and fails to reflect personal heterogeneity between patients. More relevant models are those in which cells that are injected intravenously home to various sites of the bone marrow mimicking the disseminated form of MM or when cells that are injected intratibially replicate the localized form of the disease. These intravenous and intratibial xenografts are faster and less technically challenging than the bone chip models. However, these models demonstrate the interaction of human MM cells with a normal-mouse microenvironment, which may have significant effects on the growth and drug resistance of MM cells. Again, this model lacks the aspect of personal heterogeneity between patients.

**C57BL/KaLwRij mouse model**

This model utilizes mice that spontaneously develop MM at a late age, mimics the slow pathophysiological development of MM, and demonstrates the interaction of mouse MM cells with malignant mouse stroma. However, the model is slow and time consuming, there are differences in the clinical manifestation compared to the human disease, and it lacks the aspect of patient heterogeneity.

**Summary**

Given the limitations of current models of MM, there is an urgent need to develop a new model that demonstrates the interaction of human MM cells with the human malignant bone marrow microenvironment, embodies 3-D aspects of the bone marrow niche, and takes into account personal heterogeneity between individual MM patients.

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3-D tissue-engineered bone marrow (3DTBEM) as a model for studying multiple myeloma

**Composition**

We have developed a novel patient-derived 3-D tissue-engineered bone marrow (3DTBEM) culture model autologously derived from bone marrow supernatant, MM cells, and accessory cells from the same patient. Unlike previous 3-D models based on exogenous materials, the 3DTBEM uses fibrinogen, a natural component of blood plasma, and bone marrow supernatant to develop the 3-D structure. This maximizes the similarity of the 3DTBEM to the pathophysiological bone marrow microenvironment in MM. Moreover, since the 3DTBEM is derived from the bone marrow supernatant of MM patients (Figure 3), it includes all the growth factors, enzymes, and cytokines found in the MM microenvironment, which better represents conditions in the bone marrow niche in MM.

**Demonstrating the interactions of multiple myeloma cells with their malignant microenvironment**

The bone marrow microenvironment plays a crucial role in the progression of MM. Direct interactions with bone marrow stroma, endothelial cells, and ECM—as well as cytokine-mediated indirect interactions—induce proliferation and drug resistance in MM cells and in other hematologic malignancies. Moreover, the malignant and normal microenvironment have been shown to have opposite effects on MM proliferation. While the malignant microenvironment induces proliferation of MM, the normal microenvironment reduces the proliferation of MM in vitro and in vivo. The 3DTBEM not only uses multiple types of accessory cells in addition to the MM cells, but also includes supernatant, MM cells, and accessory cells derived from the same patient. This allows a more accurate representation of the patient’s bone marrow microenvironment for studying the biological mechanisms of proliferation and drug resistance in MM. In addition, using the 3DTBEM it has been shown that stroma taken from MM patients induced more proliferation of MM cells compared to...
normal stroma. The effect of the microenvironment on drug resistance in MM cells is significantly more profound in the 3DTEBM compared to the regular 2-D culture system.40

Simulating 3-D aspects of the bone marrow niche

The bone marrow niche is a naturally occurring 3-D structure with drug and oxygen concentration gradients that are inversely proportional to the distance from blood vessels. Bone marrow cells further from the vessels have lower concentrations of drugs and oxygen, which ultimately lowers drug efficacy (Figure 4A).19

We previously found that oxygen gradients (hypoxia) that developed in the bone marrow during the progression of MM induce metastasis43,44 and drug-resistance23 in MM cells in vitro and in vivo. 2-D and 3-D models previously used for culture of MM cells that grow on the surface of a tissue culture dish, a polymer, or a bone chip, lack the aspects of drug gradients and hypoxia, which ultimately lowers drug efficacy (Figure 4A).19

The different cell types in the 3DTEBM undergo self-reorganization of the cellular structure after one week in culture, and recapitulate the polarized structure of the bone marrow niche. The 3DTEBM recapitulates drug gradients and hypoxia, which results in different patterns of proliferation and drug resistance compared to 2-D cultures.40

The different cell types in the 3DTEBM redistribute themselves according to their affinity for oxygen and, after one week, the model evolves from a homogeneous mixture to heterogeneous strata (panel B).

Reflecting individual heterogeneity in progression and response to therapy in multiple myeloma

Myeloma patients can be divided into numerous subgroups based on their genetic profile, and treatment responses vary wildly between the groups.46 In addition, it is important to note that within a given genetic subgroup, different patients show significant differences in their treatment response. Interestingly, it has been shown that response to treatment in a single patient differs depending on whether their disease status is newly diagnosed or relapsed MM. Altogether, these findings highlight the effect of individual heterogeneity on treatment outcome. With the current lack of models to support progression of primary MM cells and the extensive use of tissue-culture models based on cell lines, it is impossible to demonstrate the complexity of patients’ heterogeneity. The 3DTEBM is developed from bone marrow aspirate, with all of its autologous components (MM cells, accessory cells, and bone marrow supernatant), and can represent a specific patient at a specific stage of disease (newly diagnosed/relapsed).

Unlike any other model, the 3DTEBM is the first to support the growth of primary MM cells, both fresh and frozen, for several weeks in culture ex vivo. Thus, this novel model provides a
unique opportunity to study the biological mechanisms of proliferation, tumor-microenvironment interactions, and drug resistance in primary MM cells within their native autologous microenvironment. This is highly significant for techniques that need a large amount of patient material, such as proteomics, metabolomics, and genomics. Moreover, it provides a unique opportunity to test the effect of drugs and therapies that need the activity of other cells in the culture, such as immune-modulators, checkpoint inhibitor-αs, and mAbs, and cellular immune-therapy, such as chimeric antigen receptor T cells (Figure 5).

**3DTEBM as a novel strategy for personalized medicine in multiple myeloma**

The 3DTEBM model is now being tested in retrospective clinical trials to predict the therapeutic efficacy of drugs in individual MM patients. 3DTEBM samples developed from individual MM patients are treated with the same therapeutic regimen that the patient receives in the clinic, and the clinical response is compared with the drug-sensitivity profile obtained by the 3DTEBM ex vivo, for each patient. The method uses a high-throughput drug testing format to allow maximal efficiency, and results can be obtained within a week after establishment of the 3DTEBM culture.

The use of 3DTEBM to predict efficacy of drugs tested in clinical trials prospectively, and it will be further used to perform drug screening in the laboratory to provide the clinician with the drug-sensitivity profiles for a panel of drugs as monotherapies and as drug combinations. This will help clinicians develop better therapeutic strategies for the individual MM patient from whom the sample was initially taken (Figure 5).

**Conclusion**

3DTEBM technology provides an improved model for understanding tumor biology and drug resistance in MM in a malignant bone marrow microenvironment. It is the first model for personalized prediction of therapeutic efficacy in individual MM patients based on the biological characteristics of not only the patient’s MM cells, but also the patient’s tumor microenvironment. Studies are currently being conducted to expand the use of 3DTEBM technology to other hematologic malignancies, as well as solid tumors.

**Conflicts of interest:** Dr Azab has a pending Patent Cooperation Treaty patent application describing the 3DTEBM technology; he is owner and founder of Cellatrix LLC, a company with exclusive license for this technology. Dr Azab has received research funding from Selexys, Verastem, Karyopharm, Cleave, Glycomimetics, and Vasculox; he is the owner and founder of Targeted Therapeutics LLC.

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**References**


**Keywords:** multiple myeloma; 3-D cultures; tumor microenvironment; drug resistance; prediction of efficacy; personalized medicine
Posttreatment monitoring of lymphoma, and of other malignant tumors, has become a public health issue now that prognosis is improving. Monitoring is needed for the diagnosis of relapse, complications, and sequelae of treatment, but usually involves repeated, potentially iatrogenic, anxiety- and costly examinations. Furthermore, monitoring modalities are founded almost exclusively on habits, which differ from one center to another and may be of questionable value. Several studies have shown that monitoring has little if any impact on prognosis. In particular, unthinking routine follow-up can delay diagnosis of relapse and reduce the likelihood of initiation of optimal treatment. Follow-up with a web application used by the patient can circumvent many of these difficulties and help improve prognosis. In a phase 2 single-center trial, we found that, compared with a control group, follow-up with a web application improved survival and enabled early diagnosis of relapse and complications in lung cancer patients who responded to treatment. These results were confirmed in a randomized multicenter study. In the framework of a clinical trial, we are currently developing web application–based follow-up of lymphoma patients at high risk of relapse, with the aim of improving the poor prognosis in relapsed Hodgkin lymphoma or diffuse large B-cell lymphoma. The application is based on regular self-evaluation by the patient to detect symptoms and signs, the severity or coexistence of which is suggestive of relapse or of a complication, which prompts timely and targeted imaging as well as consultation with a specialist.

In terms of incidence, lymphoma is the sixth most frequent cancer in France, with approximately 11,000 new cases diagnosed every year. It is found predominantly in men and the elderly (median age at diagnosis is approximately 65 years), though most types can be found at all ages. Lymphoma is most commonly diagnosed following discovery of a mass in the lymph nodes. When these lymphadenopathies are deep and large, they can be revealed by the presence of various, non-specific symptoms: cough, pain, worsening of general condition, abdominal pain, etc. As such tumors are chemosensitive, treatment is mainly based on chemotherapy: chemoimmunotherapy for non-Hodgkin lymphoma and radiochemotherapy for Hodgkin lymphoma.

Thanks to the constant amelioration of diagnostic and therapeutic techniques, the prognosis of patients with lymphoma is steadily improving. Current first-line immunotherapy protocols cure about 70% of patients, but 30% to 40% of...
patients relapse, usually during the first two years after the end of treatment.\textsuperscript{2,3} In cases of relapse, patients are generally offered salvage chemotherapy followed by autotransplantation, if the patient’s age, general condition, and comorbidities permit. However, even with this optimal treatment, the prognosis of aggressive lymphoma after relapse is poor, with prolonged survival in less than 30% of patients.

Posttreatment monitoring is used to detect local or distant recurrence, identify late disease-related or treatment-related complications and sequelae, organize necessary supportive care, maintain quality of life, and diagnose a second cancer early. Standard follow-up currently comprises a clinical examination and laboratory tests every three months for two years, and then every six months for five years, plus imaging every six months. However, the value of routine imaging is questionable\textsuperscript{4-7} and has not been assessed in a randomized study. Several observational studies suggest that routine imaging can detect asymptomatic relapse in some patients, but none has demonstrated that it results in earlier detection of relapses\textsuperscript{8,9} or has a notable effect on survival. Voss et al report that in follow-up of patients (children or adolescents) with Hodgkin lymphoma in remission, most relapses are detected by onset of symptoms, clinical signs, or abnormal laboratory findings.\textsuperscript{10} Other retrospective studies reached similar conclusions.\textsuperscript{4,11-13}

Another problem is that computed tomography (CT) involves repeated exposure to radiation and hence a potentially increased risk of a second cancer.\textsuperscript{14} These imaging examinations can also generate anxiety in asymptomatic patients in remission, notably just before and pending the results of these examinations. Evaluation by imaging may result in false-positive results that prompt additional examinations, in particular biopsies, which are painful, costly, and anxiogenic.\textsuperscript{15} Such constraints would be acceptable if the relapse was detected before the clinical manifestations, thereby increasing patient survival, but one study suggests that monitoring after treatment of lymphoma using imaging procedures that involve exposure to radiation is of no benefit.\textsuperscript{16}

In a study of the usefulness of imaging for monitoring in a large cohort of patients with diffuse large B-cell lymphoma, Thompson et al concluded that routine surveillance scans worsen underlying anxiety symptoms and fear of recurrence in survivors and that strategies to minimize follow-up imaging should be investigated.\textsuperscript{17} In a recent study, El-Galaly et al reported that routine imaging detected asymptomatic relapse in only 1.7% of patients (Figure 1).\textsuperscript{18} Against this backdrop, Huntington et al found that routine imaging in asymptomatic patients with diffuse large B-cell lymphoma in remission obtained little or no clinical benefit for what is a substantial outlay.\textsuperscript{19}

Remote monitoring

A prospective study of an application developed to enable early detection of lung cancer relapses\textsuperscript{20,21} yielded encouraging results—with a sensitivity of 100%, a specificity of 89%, a positive predictive value of 81%, and a negative predictive value of 100%—by studying 11 symptoms. In the initial study, designed to establish a detection algorithm using only 6 symptoms, there was a negative predictive value of 93%.\textsuperscript{22} Relapses were on average detected 5 weeks before the scheduled follow-up examination (imaging every 3 months).\textsuperscript{23} A nonrandomized, single-center analysis of overall survival suggested a nearly 27% gain in one-year survival ($P=0.02$) compared with a control group monitored according to usual standards.\textsuperscript{22} This gain in survival was very probably related to early initiation of salvage treatment and of supportive care (Figure 2, page 396).

We compared classic follow-up by means of consultations and CT scans (every 3 to 6 months depending on the disease stage) with follow-up using the web application (Table I and Figure 3, page 396) in a phase 3 randomized trial (June 2014–2016) in 133 patients randomized to 2 arms balanced for clinical and histological characteristics. An intermediate
Application of remote monitoring to lymphomas – Denis and Solal-Céligny

Protocol analysis suggested a favorable tendency in overall survival in the patients who used the web application and were adherent (at least once a week): 62% in the standard arm versus 87% in the web application arm: $P_{(\text{log-rank})}=0.038$. This benefit was related to at least three factors:

1. Initiation of supportive care as soon as the application detects the first symptoms
2. Diagnosis of complications (pulmonary embolism, pericarditis, lung disease, sepsis…)
3. Early detection of relapse, thus facilitating initiation of salvage treatment. In our phase 3 study, 82% of patients were in good general condition at relapse (performance status 0 or 1) in the web application arm versus 35% in the standard arm ($P<0.001$). The planned treatment of this relapse was considered optimal by the investigator in 78% of patients in the web application arm versus 35% in the standard arm ($P<0.001$). (Figures 4 to 6, pages 397 and 398)

The final analysis was presented at the American Society of Clinical Oncology Annual Meeting in June 2016 and confirmed this benefit in overall survival and quality of life.

Two other studies of the remote monitoring of cancer also report increased overall survival. In the first, Bakitas et al assessed the quality of life of patients followed up using a web application for all types of cancer. This phase 3 multicenter study randomized initiation of early or delayed (by 3 months) supportive care with follow-up using an application for remote protocol analysis suggested a favorable tendency in overall survival in the patients who used the web application and were adherent (at least once a week): 62% in the standard arm versus 87% in the web application arm: $P_{(\text{log-rank})}=0.038$. This benefit was related to at least three factors:

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Figure 4. Example of relapse detection in a patient followed up using the web application. A voice change was reported and the physician received via the web application an alert concerning suspected relapse. Computed tomography revealed isolated mediastinal lymph node relapse. The patient’s general condition was maintained and stereotactic radiotherapy was performed.

Figure 5. Example of detection of pulmonary embolism. The patient’s dyspnea worsened, triggering an alert via the remote monitoring application. Prompt computed tomography angiography revealed pulmonary embolism.
monitoring of symptoms. Although the primary end point was not achieved, overall one-year survival (secondary end point) was improved by 15% in comparison with survival of patients who started follow-up by remote monitoring 3 months later.24

In the second study, Basch et al25 used remote monitoring of patients during chemotherapy for cancer (all types) in a phase 3 randomized single-center trial. The patients were seen by an oncologist before each treatment cycle, and between visits the patient did a weekly self-reported analysis of several symptoms. Here too the primary end point was quality of life, which was improved by remote monitoring. Early supportive care was initiated and overall percentage one-year survival was improved by between 6 and 14 percentage points.25

The principal limits on interpretation of these two studies are that there were various types of tumor and that the primary end point was not survival. Nonetheless, the findings tally with those of Temel et al showing that early initiation of supportive care improves overall survival in lung cancer. In this study (without remote monitoring), the patients treated first-line for lung cancer were randomized to supportive care either started at the first cycle of chemotherapy or delayed or given at the patient’s request. Although overall survival was a secondary end point, analysis showed that it improved significantly when supportive care was initiated early (Table II).22,24-27

Close clinical follow-up in general, and remote monitoring in particular, is therefore potentially beneficial in terms of overall survival. This follow-up expedites supportive care as soon as the first symptoms appear and, for certain applications like ours, early detection of relapse and complications.

Remote monitoring in lymphoma

No prospective trial has assessed remote monitoring by analysis of lymphoma symptoms, and we have seen that the usefulness of CT or positron emission tomography is unproven...
In follow-up of lymphoma. Yet should we leave patients to their own devices between two medical appointments? Or would early detection of relapse and prompt initiation of supportive care be beneficial?

Reinforcing clinical follow-up in this indication, in which routine imaging is of unproven benefit (notably by means of remote monitoring completed by the patient), can be advantageous in terms of efficacy and early treatment. In lymphoma, up to 40% of patients relapse early (within 2 to 3 years), symptomatically in the vast majority of cases (less than 2% of asymptomatic relapses are detected by imaging). Lastly, current follow-up by CT every 6 months generates costs and exposure to radiation for a rather limited benefit and is performed in patients who have been symptomatic for several weeks.

As in lung cancer, reinforced clinical follow-up can lead to early detection of relapses via the monitoring of all frequent, clinically significant complications in patients with serious illness (sepsis and other immunosuppression-related complications, thromboembolic disease, late iatrogenic conditions, etc). However, against a backdrop of increasing medical needs and limited recourse to health systems, plus medical shortages and strict controls on health-care spending, reinforced clinical follow-up can only be envisioned through the implementation of means, if effective, that are as time- and cost-efficient as possible. This is why patient involvement and the use of telehealth technology are highly relevant today, as they save the patient unnecessary trips and examinations.

We have developed a score specific to lymphoma based on the dynamics and association of symptoms and clinical and biochemical signs that alert physicians to possible relapse. The variables studied are: changes in weight and appetite, breathlessness, asthenia, generalized pruritus, pain, fever, hyperhidrosis, skin lesions, subcutaneous or lymph nodules, edema, depression, hemoglobin <12 g/dL, and lactate dehydrogenase >2× normal levels. Patients report these clinical symptoms every 1 to 2 weeks, using their smartphone (Figure 7) or computer (via the Internet), and the variables are analyzed by software that determines the probability of relapse or of significant complications. The attending physician therefore receives an alert and can schedule a battery of tests (Figure 6 and Table III).

In our multicenter study of the web application we have developed, standard follow-up was offered to all patients (medical appointment every 3 months, CT every 6 months). The primary end point was the number of events detected using the application and confirmed by a medical examination performed outside standard follow-up.

Follow-up using our web application improved survival compared with usual, routine follow-up, in a phase 2 trial and then in a phase 3 randomized trial in patients with lung cancer that...
responded to treatment. This improvement was linked to early initiation of supportive care and to the heightened possibility of starting a treatment specific to the relapse. Armed with these results, we developed a web application for the follow-up of lymphoma patients at high risk of relapse. This should increase the number of relapsing patients who receive effective chemotherapy, such as paxitron, in aggressive non-Hodgkin lymphoma.

Caution should, however, be exercised in disseminating monitoring methods:

- their efficacy should first be confirmed in rigorous clinical trials in the main types of tumors, which differ substantially in progression;
- they should be provided to as many patients as possible along with suitable explanations for use; and
- hospital organizations should expedite rapid and rigorous data management.

**Conclusion**

We envision a large number of other uses for monitoring by web application, including screening for complications like febrile neutropenia after chemotherapy in phase 2 trials using an application that we have developed, follow-up of patients included in a clinical trial (notably early phase trials), and postoperative monitoring after outpatient surgery. As in other areas, the digital world has much to offer in optimizing the management of cancer patients.

**References**


**Keywords:** remote monitoring; lymphoma; telehealth; self-reported analysis; application; smartphone; computer; optimized management
The first source of big data, including electronic medical records, claims and billing data, imaging data, and pharmacy data, is from providers and payers. The second source is omics, including genomics, proteomics, and metabolomics. The third comes from patients and nonproviders, i.e., data from smartphone and Internet activities, sensors, and monitoring tools. In the future, about two-thirds of all medical-related data will come from patient-created sources.

Big data, technology, and the changing future of medicine

by M. Bauer, Germany

Michael Bauer, MD, PhD, is Professor of Psychiatry and Executive Chair of the Department of Psychiatry and Psychotherapy at the Medical Faculty, Technische Universität Dresden, in Dresden, Germany, where he is also Physician-in-Chief at the Psychiatric Hospital and Outpatient Clinics of the University Hospital Carl Gustav Carus. He received his medical degree from Freie Universität Berlin School of Medicine and a PhD in molecular biology and biochemistry from Freie Universität Berlin. He has authored more than 400 articles published in peer-reviewed journals. He is Editor-in-Chief of the International Journal of Bipolar Disorders and Pharmacopsychiatry. His research interests include: prediction, response, and outcome in psychopharmacology; the neurobiology of mood disorders by applying methods of functional neuroimaging, neuroendocrinology, and genetics; and the development of new technologies for longitudinal, digital assessments and phenotyping in psychiatry. He introduced new approaches to self-management and monitoring, opening up new treatment horizons for sufferers of bipolar disorders.

The future of medicine will be enabled by big data. Today, the amount of medical data in the world is massive and growing quickly, a result of the convergence of evolutionary changes in many technologies over the past decades. With the promise of data-driven knowledge and decision-making, innovative big-data projects are ongoing in every area of medicine. For big-data projects to succeed, physician expertise and participation is required throughout every phase. This article will review some of the fundamental technology behind the rise of big data, and discuss the impact of big data on medicine in the future.

Medicographia. 2016;38:401-409

The future of medicine offers individualized care tailored to the patient, integrated care delivery, intelligent decision-support systems for physicians, an increasing emphasis on prevention, and a systems-oriented rather than reductionist approach to the understanding of disease. All of these goals are enabled by big data. In 2013, the amount of digital medical data in the world was estimated at 153 exabytes (1 exabyte = 10^18 bytes), an amount so large it is hard to fathom. For perspective, consider that the estimate of 5 exabytes would store all the words ever spoken by human beings.¹

¹ Medicographia. 2016;38:401-409
Although there are many definitions, big data is large, heterogeneous, from many sources, and may arrive in real-time. The scale, speed, and complexity of big data make it challenging to process, analyze, and extract information. Big data is too massive for humans to understand without the assistance of computer models.

There are three primary sources of big data in medicine. The first source of big data, including electronic medical records (EMRs), claims and billing data, imaging data, and pharmacy data, is providers and payers. The second source is omics, including genomics, proteomics, and metabolomics. The third is patients and nonproviders, i.e., data from smartphone and Internet activities, sensors, and monitoring tools. In the future, about two-thirds of all medical-related data will come from patient-created sources.

Not only is the amount of digital medical data massive today, it is projected to grow at 48% a year through to 2020. There are many examples of why digital health-care data is growing so quickly. The yearly amount of data per patient created in an EMR is about 80 MB, with 95% of this being imaging data. The UK National Health Service stores 1 million new images every day in its database. The storage required for imaging data is increasing as new imaging techniques offer higher resolution images with 3 or 4 dimensions. Genomic data is starting to be collected and requires about 50 times more storage per patient than imaging data. Bedside monitoring equipment used in a hospital intensive care unit records between 1000-2000 data points per second per patient (Figure 1). In 2015, 4.9 million patients used prescribed remote home monitoring, most for cardiac care. Growth to 36.1 million patients is expected by 2020. Patients are starting to use health apps. The 165,000 health apps available for Android and Apple smartphones (Figure 2) are forecast to be downloaded 1.7 billion times by 2017.

The promise of data-driven knowledge and decision-making has completely changed the twentieth century paradigm of a focus on technology to a twenty-first century focus on data. Now, technologies are being designed around data rather than data being designed around technologies. Although digital environments in medicine, such as EMR everywhere and radiology systems that provide “images at the right place at the right time,” are now taken for granted, these are relatively recent advances. The dawning era of big data was enabled by a confluence of technological progress in many fields that evolved over decades.

Technologies contributing to the growth of big data in medicine

To better understand the scope of big data in medicine, we need to briefly look back on technological progress over the last few decades.
Over the past decades there were dramatic improvements in the number of bits that could be stored on a hard drive. Since the introduction of hard drives in 1956, the density of information stored has increased from 2000 bits to 100 billion bits (gigabits). With the exponential increase in hard disk capacity came a shrinking product footprint and a fall in prices. To-day, cloud providers make the storage capacity of thousands of hard disks available on demand to allow very large databases to be quickly assembled for short or long periods of time.

**Network connectivity**

Big data in medicine requires network connectivity with other locations, physicians, disease registries, scientists, literature depositories, and patients—a fundamental part of big data in medicine. The number of objects connected to the Internet has grown and continues to grow rapidly, from 10 billion objects in 2013 to a predicted 50 to 100 billion objects by 2020.

**Microprocessor computing power**

The foundation for the digital explosion, including big data in medicine, is the dramatic improvement in performance and decrease in price of microprocessors. The doubling of the number of transistors on an integrated circuit about every 2 years since the mid-1960s is a historic engineering feat predicted by Gordon Moore. Going from tens of transistors to over a billion on a single microchip has resulted in exponential improvements in microprocessor speed, enabling many types of general purpose and specialized processors.

The need to analyze, process, and visualize big data requires more than the raw performance in a single microprocessor. Big data requires that analyses be split into pieces and completed in parallel using multiple microprocessors. The architecture for the big-data era will be based on multicore processors (chips with multiple processing cores) (Figure 3). The future will see an increasing number of cores in each multicore processor, and more multicore processors working concurrently. Research will continue to address the challenges of power delivery, thermal constraints, and reliability in multicore processors.

**Data storage**

An immediate result of the advances in microprocessor technology was an enormous increase in the amount of data being generated and stored. A typical 500 bed hospital requires more than 50 petabytes ($50 \times 10^{15}$) of data storage. Over the past decades there were dramatic improvements in the number of bits that could be stored on a hard drive. Since the introduction of hard drives in 1956, the density of information stored has increased from 2000 bits to 100 billion bits (gigabits). With the exponential increase in hard disk capacity came a shrinking product footprint and a fall in prices. To-day, cloud providers make the storage capacity of thousands of hard disks available on demand to allow very large databases to be quickly assembled for short or long periods of time.

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◆ Mobile access
Mobile technology (cellular and wireless) has triggered the start of remote patient monitoring and remote disease management, and patient-generated data will be an increasing component of big data. Mobile data traffic has grown almost 4000-fold over the past 10 years, and mobile traffic volumes are expected to increase by several hundredfold over the next decade. The comparatively low cost of mobile technology allowed the rapid creation of networks in developing economies, bringing Internet access to millions. In 2015, 95% of the world’s population was covered by a 2G mobile cellular network, and 47% by 3G mobile broadband. The global percentage of mobile subscribers in 2015 was 63% or 4.7 billion unique subscribers. About 43% of total handsets in 2015 were smartphones, with an expected increase to 50% by 2020 (Figure 2).

A vision of the future is of a networked society with unconstrained access anywhere, anytime, to anyone or anything. Mobile technology will continue to evolve to address challenges related to the simultaneous need for high-capacity, high-reliability, and very low latency.

◆ DNA sequencing
Few new technologies have exceeded the pace of change in microprocessors, but advances in DNA sequencing technology have been revolutionary (Figure 5). The direct cost of sequencing a human-sized genome has dropped from about $95 million in 2001 to $1245 in 2015. Next-generation sequencing technology uses massively parallel analysis, producing millions of sequence reads in a single run. These technological advancements have increased the number of scientists and laboratories that are able to participate in genet- ric research, allowed the investigation of an unprecedented number and variety of biological questions on a genome-wide scale, and provided results at an unimaginable speed. The new sequencing techniques create massive amounts of data, and pose challenges to both software algorithms and statistics used to analyze the data. There are also many challenges for implementation of next generation sequencing in the clinical workplace. The new technology has already led to a huge increase in the number of human genetics studies, created new knowledge about the relation between DNA and disease, and triggered the growth of megaconsortiums to sample millions of individuals.

A recent example of big data in genetics research is the work by the international Consortium on Lithium Genetics (www. ConLiGen.org) assembling, to date, the largest genome-wide association studies on response to lithium (a mainstay in the pharmacological treatment of bipolar disorder, a drug with a largely unknown mechanism of action) in bipolar disorder, totaling over 2500 individuals. The consortium, involving two French centers, in Paris and Creteil, recently presented genome-wide significant evidence of association between lithium response and common genetic variants on chromosome 21. The genetic region associated with response contained two long noncoding ribonucleic acid genes, which are increasingly appreciated as important regulators of gene expression, particularly in the brain. This study suggests that a better understanding of drug mechanisms and response can be achieved through international cooperative efforts that link clinical expertise with large-scale genomics.

◆ Sensors
Remote monitoring of patients at home has become feasible in recent years due to sensing technologies that provide passive data gathering. Modern embedded sensors are miniaturized, inexpensive, lightweight, and ultra-low power, and multiple sensors are routinely combined in a single device. For example, a modern smartphone includes sensors to measure internal motion (accelerometer), ambient light, angular velocity (gyroscopic), magnetic fields for orientation (magnetometer), activity in relation to altitude (barometer), and outdoor localization (GPS [global positioning system]) (Figure 2).

Many sensors are available for physiological measurements, including heart rate, electroencephalography, respiration, blood pressure, and skin conductivity. Sensors for patient monitoring are also placed in homes on walls and floors. Wearable sensor technologies may be preferable for some patients. Wearables include devices worn under or over clothing, e-tex-
the scope of big data–related projects in medicine. A diverse sample of big data studies is shown in Table I. Projects require the collaboration of people with a wide range of expertise. Successful analysis of medical big data requires the active participation of physicians, statisticians, biologists, software engineers, mechanical engineers, network security experts, and project managers. Procedures and quality standards are required for every aspect of a project. More international standards for technological protocols and automatic data interpretation are needed.

Challenges of big data

Using big data in medicine is not easy. Every step in the acquisition, processing, cleaning, analysis, and interpretation of big data is difficult (Figure 6), and projects require the collaboration of people with a wide range of expertise. Successful analysis of medical big data requires the active participation of physicians, statisticians, biologists, software engineers, mechanical engineers, network security experts, and project managers. Procedures and quality standards are required for every aspect of a project. More international standards for technological protocols and automatic data interpretation are needed.

There are also many implementation issues, both technical and clinical, to incorporate, process, and interpret the influx of data into clinical practice, eg, from continuous patient monitoring. The differences between the data generated by the diverse technologies contribute to the analytical challenges for the secondary use of medical data in research. Big data includes structured data (such as in a form, spreadsheet, or relational database), unstructured data (text, imaging, audio), and semi-structured data (XML documents). About 80% of the data in health care is unstructured, and there are tremendous challenges trying to process the diverse medical vocabulary in natural language text. Big data is very complex or high-dimensional, and it may have huge numbers of parameters available for each patient, huge numbers of patients, or both. Most big data are not generated for or from research, and data collected from different sources are of different quality. Data quality is related to type, biases, and provenance or

<table>
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<tr>
<th>Field of medicine</th>
<th>Project</th>
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<td>Clinical chemistry</td>
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<td>Pathology</td>
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Table I. The scope of big data–related projects in medicine.

with a wearable sensor will soon be used to measure patient adherence. Novel materials and device fabrication strategies will expand the role of ingestible sensors to include controlled release of drugs and detection and treatment of abnormalities in the gastrointestinal tract. Many innovative big-data projects are ongoing in every area of medicine. A diverse sample of big data studies is shown in Table I. Projects require the collaboration of people with a wide range of expertise. Successful analysis of medical big data requires the active participation of physicians, statisticians, biologists, software engineers, mechanical engineers, network security experts, and project managers. Procedures and quality standards are required for every aspect of a project. More international standards for technological protocols and automatic data interpretation are needed.

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ownership trail, and data quality is reduced by missing values, inconsistency, untimeliness, or poor error-handling. There are many biases and quality issues in both EMR and claims data. Data obtained from Internet sources, such as social media, are not representative of the entire population, but only of the self-selected group who use the specific site. The analysis of very large observational data may be associated with many spurious findings, and the analysis will primarily measure correlation not causality. If the analysis of big data is based on classic statistical methods, underlying statistical assumptions are likely violated. New analytical techniques combining statistical (model-based focus on variability) and algorithmic (data mining for patterns and rules) approaches are being developed for big data. Investigation of medical big data requires the active participation of those with subject matter expertise.

Despite the dramatic changes in technology, human processing capacity remains roughly the same. Applications that use big data must address human abilities related to multitasking, data visualization, and cognitive processing. The new field of visual analytics is working to improve system interfaces by combining the power of computer processing with the outstanding human ability to recognize visual patterns. The sensory overload (Figure 7) and alarm fatigue due to excessive audible and visual alerts from a myriad of physiologic monitors in a hospital demonstrate the importance of the human interface. One of the greatest challenges with big data is not just a question of throwing ever increasing amounts of processing power at the problem of analysis. A holistic, start-to-finish approach that considers data acquisition (panel A), processing (panel B), cleaning, analysis, and interpretation is essential to obtain the best from big data. The mixture of different types of data—structured, unstructured, and semi-structured data—and multiparameter monitoring in health care make analysis of medical big data complex. Most big data (about 80%) in health care are of the unstructured variety.

Figure 6. Challenges of acquiring and analyzing medical big data.
Making the best of use of medical big data is not just a question of throwing ever increasing amounts of processing power at the problem of analysis. A holistic, start-to-finish approach that considers data acquisition (panel A), processing (panel B), cleaning, analysis, and interpretation is essential to obtain the best from big data. The mixture of different types of data—structured, unstructured, and semi-structured data—and multiparameter monitoring in health care make analysis of medical big data complex. Most big data (about 80%) in health care are of the unstructured variety.

Figure 7. Caught in the data deluge...
It’s good to have a lot of data, but you don’t want to end up drowning either. With medical big data, as in other domains, quantity does not equal quality. Much depends on factors such as data source, condition, bias, provenance, completeness, consistency, and timeliness. New analytical techniques, which combine statistical and algorithmic approaches, will help bring out the best in big data.
data is to create useful and useable tools for clinical medicine that provide the information that physicians want to know in a clear and timely manner.

Ethics of big data

The use of big data for medical research also poses unprecedented ethical challenges. These include questions related to individual privacy, data confidentiality, informed consent, involvement of commercial organizations, reuse of data, reidentification of deidentified data, differences in international privacy regulations, and changing societal attitudes towards public and private data. Big data projects are often distributed across multiple countries, making issues of data management, privacy, and consent more complex. Cloud storage in unknown countries complicates legal jurisdiction. Privacy laws vary from country to country, and many countries have not addressed the impact of modern technology on existing regulations. There are many problems related to data created by patients. Patients may incorrectly assume that all medical privacy laws apply to commercial Internet companies, downloaded health apps, or data provided to health websites.

References


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