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Molecular targeted therapies for indolent lymphomas: where are we?

During the last decade the development of new drugs for the treatment of hematologic malignancies has become really promising. This innovative drug development is based on the translation into biochemical pharmacology of specific alterations of biological functions affecting tumour cells1. One of the most important examples is imatinib: it showed that it was possible to nullify the pathognomic genetic lesion of chronic myelogenous leukemia (CML). Drugs targeting unique disease-specific pathways have also found potential applicability in treating malignancies such as CD20-positive non-Hodgkin's lymphoma (rituximab), follicular lymphoma (Bcl-2-targeted agents[2-6]) and other B-cell neoplasms (splenic tyrosine kinase [Syk] inhibitors;[7-9] IkB kinase inhibitors[10]).

Rituximab, whose initial employ in treatment of lymphomas dates to more than 10 years ago, is perhaps the most exploited and well-known "molecular-targeted" drug in treatment of indolent lymphoproliferative disorders. Its use in first-line therapy of indolent $CD20^+$ lymphomas should not be discussed: nowadays, more concerns are about the most effective schedules or combination regimens for maintenance, above all in follicular lymphoma. A recent meta-analysis[11] suggested that maintenance therapy with rituximab, either as four weekly infusions every 6 months or as a single infusion every 2-3 months, should be added to standard therapy for patients with relapsed or refractory follicular lymphoma after successful induction therapy, to improve progression-free and overall survival. In contrast, previously untreated patients seem not to acquire the same benefit from the maintenance treatment (i.e. it prolongs the time to progression but does not prolong survival).[12-13]

The data recently showed at 2008 ASH Meeting, coming from the clinical phase III studies named CLL8 and REACH, definitely support the employment of rituximab in addition to chemotherapy (the standard regimen being fludarabine plus cyclophosphamide) in patients affected by chronic lymphocytic leukaemia, (the most common indolent lymphoproliferative disorder) both in first and in second line treatment, achieving superiority in terms of progression free survival.

Since rituximab is a chimeric antibody, there is a need to develop fully humanised antibodies, in order to minimise infusion reactions and eliminate the development of human antibodies against the drug itself. In such a perspec-

tive, new anti-CD20 antibodies named ofatumumab,[14] IMMU-106 (hA20),[15] and GA101[16] have been developed and used in treatment of recurrent lymphomas, with promising results in phase I/II clinical studies.

Further clinical evaluation of antibodies has been largely based on the knowledge of antigen expression on the surface of lymphoma cells and has led to the development of antibodies against many surface molecules: CD22 (unconjugated epratuzumab and calicheamicin conjugated CMC-544 [inotuzumab ozogamicin]), CD80 (galiximab), CD52 (alemtuzumab), CD23 (IDEC-152 [lumiliximab]) are the surface molecules against which monoclonal antibodies have been directed and employed in clinical studies with patient affected by indolent lymphomas and/or CLL.

Epratuzumab[17] has induced responses in 24% of patients with follicular lymphoma and in 15% of patients with recurrent diffuse large-B-cell lymphoma, without dose-limiting toxic effects.

Galiximab[18] achieved positive responses in 15% of patients affected by recurrent follicular non-Hodgkin's lymphoma, some of which occurred as late as a year after treatment.

Alemtuzumab[19-20] has been approved by the FDA for the treatment of chronic lymphocytic leukemia. About 30% of patients with CLL whose disease progressed after they received alkylating agents and fludarabine have had a response to alemtuzumab. When alemtuzumab is used as the initial therapy in CLL, the response rate is about 80%.

Lumiliximab[21] showed he ability to induce apoptosis of CLL cells *in vitro*; in a phase II trial, CLL patients who received lumiliximab and FCR attained higher response rates (ORR 71%, CR 48% and PR/PRu 23%) than historical FCR-only controls. A phase III trial comparing FCR with and without lumiliximab is ongoing.

A chance to improve the effect of monoclonal antibodies is offered by radioimmunotherapy (RIT), combining monoclonal antibodies with radionuclides. Two different murine anti-CD20 antibodies (Y-^{90}ibritumomab and ^{131}I tositumomab) have been approved for the treatment of rituximab-resistant, relapsed or refractory indolent lymphomas. Y-^{90}ibritumomab (Zevalin®) achieved higher ORR and CR rates than rituximab in relapsed/refractory FL, but time to progression (TTP) and duration of remission (DOR) were not statistically significant.[22-23] ^{131}I tositumomab (Bexxar®) achieved ORR of 60-70% in heavily pretreated patients.[24] Besides its use in treating refractory lymphomas, radioimmunotherapy also seems promising in consolidation approaches after chemoimmunotherapy, and it could represent a great chance to replace rituximab as maintenance treatment in indolent lymphomas.

So far only in the context of experimental studies, some murine and humanized forms of anti-CD22 antibodies have also been used conjugated with different radionuclides, among which Y-90 is the most promising: this kind of CD22-RIT has been used in 16 patients with previously treated NHL,[25] obtaining an ORR of 62% (75% in indolent NHL and 50% in aggressive NHL) with 25% CR/CRu rates. Three weekly infusions were feasible in this population with minimal toxicity.

Among the best examples of target-therapies in lymphomas, a place is occupied by immunotoxins, consisting of protein toxins connected to cell binding ligands including monoclonal antibodies and growth factors, have been developed for several decades to target hematologic malignancies. Protein toxins from either plants or bacteria are extremely potent based on their enzymatic inhibition of protein synthesis and induction of apoptosis. Plant toxins, particularly ricin, are useful for chemically conjugating to monoclonal antibodies, and have shown clinical activity in several types of lymphoma and leukemia; unfortunate-

ly, their dose is generally limited by the possible onset of vascular leak syndrome. Denileukin diftitox[26](the only approved drug containing a protein toxin) has shown efficacy in cutaneous T-cell lymphoma, chronic lymphocytic leukemia, and non-Hodgkin's lymphoma. Recombinant immunotoxin BL22[27] is an anti-CD22 Fv fragment fused to truncated Pseudomonas exotoxin; it induces complete remissions in a high percentage of patients with chemoresistant hairy cell leukemia.

Proteasome inhibitors are an exciting class of agents that have been used in multiple myeloma and mantle cell lymphoma. Proteasome inhibitors disrupt the pathways involved in the pathogenesis of non-Hodgkin's lymphoma, and preclinical models showed sensitivity in lymphoma cell lines to proteasome inhibitors.[28-29] Bortezomib and carfilzomib are currently under study for indolent lymphomas.[30-33] A 2006 study by de Vos and colleagues compared weekly and twice-weekly schedules of bortezomib in combination with rituximab in a group of patients who were sensitive to rituximab.[34] Eighty-six percent of the patients had follicular lymphoma. Toxicities differed between the two arms, with the twice-weekly arm experiencing more grade 3 adverse events (54% *vs.* 35%), but the outcomes were similar. However, without a rituximab-alone arm, it is hard to determine the degree to which the bortezomib is contributing to these response rates. As there is not yet a good control population for the patients who have relapsed follicular lymphoma, this combination is now in a randomized trial comparing combination bortezomib and rituximab to rituximab alone.

Nowadays, bortzomib as single-agent therapy seems even more powerful in treating patients with relapsed or refractory mantle cell lymphoma, achieving lengthy responses and notable survival, with considerable TTP and TTNT in responding patients, suggesting substantial clinical benefit.[35]

In addition to proteasome inhibitors, the immunomodulatory drugs (IMiDs), such as lenalidomide, have the potential to improve outcomes for patients with NHL. These drugs inhibit cell growth and proliferation by several mechanisms, including blocking the effect of growth factors and stimulating T cells and natural killer cells.[36-37] Lenalidomide is particularly effective in lymphoproliferative disorders such as multiple myeloma, and it is active in patients with various forms of NHL, with a favourable side-effect profile. The most interesting results seem to be achieved if employed in mantle cell lymphoma, with a high response rate reached even in patients with relapsed or refractory disease.[38] Complimentary clinical and pharmacological features suggest that lenalidomide may be effective when combined with monoclonal antibodies. Ongoing and future studies will provide further information.

During the past several decades, understanding the many complex pathways of cell survival provided a fertile arena for formulating new strategies to target these pathways in human cancer. It was demonstrated (1) the existence of pro- and anti-apoptotic relatives of Bcl2 which, through specific interactions, modulate the balance of pro- and anti-apoptotic fates; (2) the existence of at least two distinct pathways leading to apoptosis in mammalian cells, one involving the mitochondria (the intrinsic pathway) and other involving cell-surface death receptors (the extrinsic pathway).

The fruit of the research on Bcl-2 and its kinases have led to new therapeutic strategies, some of which have been tested in clinical trials in humans.

The first of these is an antisense oligonucleotide targeting Bcl-2 mRNA, Oblimersen:[39-40] it downregulates BCL-2 in a concentration- and time-dependent manner. It showed an anti-

tumor efficacy in CLL patients especially in association with chemotherapy (fludarabine plus cyclophosphamide) with higher CR and PR and prolonged time to relapse. Superior benefits were obtained in chemosensitive patients' group.[41]

Obatoclax mesylate (GX-15-070) is a pan Bcl-2 inhibitor: it inhibits several anti-apoptotic BCL-2 family proteins including Bcl-XL, Bcl-2, Bcl-W, Bcl-B, A-1 and MCL-1, and has got a pro-apoptotic function through activation of BAX and BAK. It induces apoptosis in human CLL cells treated ex-vivo and has an additive effect in association with fludarabine and chlorambucil. Dose limiting toxicities were neurological and temporally associated with drug infusion.[42-43]

Gossypol (AT-101) is an orally available compound, which binds to the BH3-binding grooves of Bcl-2, Bcl-XL and MCL-1, displacing BH3-peptides. It promotes an allosteric conformational change in Bcl-2 and loss of mitochondrial membrane potential in a BAX/BAK-independent way.[44] Gossypol induces cytochrome c release from mitochondria and activation of several caspase. Its ability to improve the efficacy of a combination chemotherapy regimen (CHOP) in xenograft models of lymphoproliferative disorders has been recently demonstrated.[45]

Other compounds can induce a phenotypic conversion that turns Bcl-2 into a pro-apoptotic protein, by binding to a large unstructured loop between the Bcl-2 BH3 and BH4 domains. One of these compounds mimics the NUR77 (nuclear receptor) activity on Bcl-2 and consists in a 9 amino-acid peptide, named NuBCP-9. The conformational change in Bcl-2 interferes with the binding and sequestering of BH3-only proteins, turning them into BH3-like proteins that inhibit anti-apoptotic peptide Bcl-XL inducing a BAX/BAK-via apoptosis.[46]

ABT-263 also binds with high affinity to several anti-apoptotic Bcl-2 family proteins (as a first generation inhibitor named ABT-737 did); ABT-263 was administered orally in both indolent and aggressive lymphomas: some patients achieved a significant tumour volume reduction.[47]

Another way to stimulate the apoptosis is the activation of extrinsic pathways through the development of TRAIL receptor agonists. These effective agents are antibodies targeting DR4 (mapatumumab[48]) and DR5 (lexatumumab[49]) proteins, that induce apoptosis in cancer cells but not in normal cells. Antibodies have the advantage of a long half-life and additional mechanism for cell-killing through antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. In a phase II study with mapatumumab in relapsed and refractory NHL relevant clinical responses were reported, including a CR reached in a patient affected by follicular lymphoma.[50]

A plethora of novel small molecules, each of them with its own distinct pharmacology, represents a potentially important strategy for modulating the threshold required to induce programmed cell death, in synergy with other DNA-damaging agents, and it offers now a promising chance to cure some patients with lymphoproliferative disorders.

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