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Treatment of Acute Lymphoblastic Leukaemia
A New Era

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Contents

Abstract

Acute lymphocytic leukaemia (ALL) is a heterogeneous group of disorders that result from the clonal proliferation and expansion of malignant lymphoid cells in the bone marrow, blood and other organs. Distinct clinicopathological ALL entities have been identified, resulting in the adoption of risk-oriented treatment approaches. Advances in ALL therapy have led to long-term survival rates of >80% in children. However, only ≈30–40% of adults achieve long-term disease-free survival. Contemporary ALL treatment programmes include induction, intensified consolidation, maintenance phases and CNS prophylaxis. The optimal treatment of Philadelphia chromosome-positive patients requires the addition of BCR-ABL tyrosine kinase inhibitors, such as imatinib, whereas allogeneic stem-cell transplantation remains the preferred approach for high-risk patients in first remission. Since only ≈38% of adult ALL patients are free of disease 5 years after diagnosis and the outcome of salvage chemotherapy is very poor (complete remission rates of 20–30%, median survival of 3–6 months), novel

agents are desperately required. Of those currently in clinical studies, the outlook for sphingosomal vincristine, pegylated asparaginase (pegaspargase), liposomal annamycin, ABT-751, pemetrexed, talotrexin, nelarabine and the novel BCR-ABL kinase inhibitors is discussed.

Acute lymphocytic leukaemia (ALL) is a heterogeneous group of disorders that result from the clonal proliferation and expansion of malignant lymphoid cells in the bone marrow, blood and other organs.[1] As our understanding of the pathophysiology of ALL has deepened, distinct clinicopathological ALL entities have been identified as being associated with different prognoses and responses to therapy. This has led to the development of risk-oriented treatment approaches.[2] Advances in ALL therapy have led to long-term survival rates of >80% in children.[3] Complete remission (CR) rates comparable to those in children can be achieved in adults by adapting paediatric ALL treatments; however, only ≈30–40% of adults achieve long-term disease-free survival (DFS).[2]

Contemporary ALL treatment programmes are constructed on a template that, in general, includes induction, intensified consolidation and maintenance phases, with CNS prophylaxis being a fourth component that accompanies both induction and consolidation. Remission induction therapeutic schemas, which aim at inducing CR, incorporate a combination of chemotherapeutic agents that are moulded into various dosage schedules, which usually include vincristine (VCR), anthracyclines and corticosteroids as a backbone. Intensification of induction with additional agents has positively influenced the outcome in some ALL subsets, e.g. T-cell lineage ALL (cyclophosphamide, cytarabine) and mature B-cell ALL (fractionated doses of cyclophosphamide, high-dose methotrexate [HDMTX], rituximab).[1,4-9] Compared with the earliest and less-intense VCR, doxorubicin and dexamethasone (VAD) programme, the more intensive regimen of hyperfractionated cyclophosphamide, VCR, doxorubicin and dexamethasone (hyper-CVAD) was associated with significantly better CR, CR duration and survival.[10] Analogous results regarding CR rates (>90%) were also observed with an induction schema, which was recently instituted by the MRC UKALL XII/ECOG (Medical Research Council UK Acute Lymphocytic Leukaemia XII/Eastern Cooperative Oncology Group) trial.[11] Overall, there has been a CR rate of ≈80% in adult ALL with a 5-year survival rate of 30–40%.[2,10,12]

Intensification therapy refers to a modified induction modality, high-dose chemotherapy (high-dose cytarabine, HDMTX), stem-cell transplantation (SCT) or even the use of new agents, with the objective to eliminate residual leukaemia, often undetectable by conventional methods, as well as to overcome resistance. Overall, in adult ALL, intensification therapy prolongs leukaemia-free survival, with allogeneic SCT from sibling or unrelated donors or autologous SCT being the preferred approach as an intensive post-induction treatment in patients with high-risk characteristics. Traditionally, maintenance therapy is given for 2–3 years and is based on mercaptopurine and methotrexate (MTX). Omission, or shortening of maintenance therapy, worsens outcome.[13] Patients with mature B-cell ALL do not require maintenance because they attain high-cure rates with dose-intensive, short-term chemotherapy and relapses beyond the first year in remission are rare.[2] The optimal treatment of Philadelphia chromosome-positive (Ph+) patients requires the addition of BCR-ABL tyrosine kinase inhibitors, such as imatinib, or the novel inhibitors discussed in section 5.

Approximately 38% of ALL patients are free of disease 5 years after diagnosis; however, the overall survival at 5 years after relapse is 7%.[14] Although SCT is considered superior to chemotherapy when disease relapses, with long-term DFS rates of 20–40%, its feasibility is often very limited.[15,16] Moreover, the outcome of salvage chemotherapy remains very poor, with CR rates reported to be 20–30% and a median survival ranging from 3 to 6 months. In general, salvage programmes are patterned in accordance with frontline therapies;[17-20] however, recently, newer agents have been introduced in the therapeutic arena and some of them have already shown promising results (e.g. clofara-

bine[21,22] and nelarabine[23]). The dismal outcome for patients in the salvage setting necessitates the development of new agents. Of those currently in clinical studies, sphingosomal VCR, liposomal annamycin, pegylated asparaginase (pegaspargase, Oncospar® [1]), ABT-751, pemetrexed (LY-231514, Alimta®), talotrexin (PT-523, Talvesta™), as well as nelarabine and the novel BCR-ABL kinase inhibitors, are briefly reviewed. Some of these agents were selected because they were most recently developed and therefore only limited bibliographical information currently exists; available preclinical data are, therefore, described in detail in an attempt to justify their incorporation in clinical trials for ALL. Selection of the rest was based on the fact that they are currently in later stages of clinical development and have proven to be promising assets in the therapy of ALL.

1. New Formulations of Old Drugs

1.1 Liposomal Agents

The liposomal formulation of a chemotherapeutic agent alters its pharmacological properties and, thus, the toxicity of the active compound. In general, reduced toxicity and increased efficacy is observed. Antimicrotubule agents and topoisomerase II inhibitors have both been encapsulated in liposomes (i.e. liposomal VCR, liposomal annamycin, and liposomal daunorubicin [DaunoXome®]),[24,25] and have been successfully used in clinical trials involving ALL patients.

1.2 Sphingosomal Vincristine

The vinca alkaloid agent VCR is an important drug in the treatment of ALL. It causes cytotoxicity by binding to tubulin and thereby inducing depolymerisation of microtubules and metaphase arrest.[26,27] The extent of cellular apoptosis caused by VCR *in vitro* is concentration and exposure-time dependent;[28-30] however, a dose increase >2.0mg *in vivo* is not safe because at higher doses the drug disrupts neuronal axonal microtubules and causes neurotoxicity (peripheral neuropathy, intestinal toxicity).[31] Moreover, conventional (aqueous) VCR has a poor bioavailability and following intravenous (IV) administration it is rapidly eliminated from the circulation (half-life [$t_{1/2}$] = 1.36 hours), has a low-plasma area under the plasma concentration-time curve (AUC) [0.59 μg/h/mL] and a large volume of distribution (145mL).[32] These unfavourable pharmacokinetic properties can be altered by encapsulating VCR into liposomes.[33,34] Distearoylphosphatidylcholine/cholesterol (DSPC/chol) and sphingomyelin/cholesterol (SM/chol or shingosomal) VCR are the two most commonly used liposomal formulations of VCR in preclinical and clinical studies.

Sphingosomal VCR is therapeutically superior to DSPC/chol VCR in preclinical tumour models,[35,36] because its chemical configuration makes it less susceptible to acid hydrolysis and therefore less 'leaky' for the contained VCR. The entrapment of VCR into the SM/chol liposome greatly prolongs its $t_{1/2}$ (to 6.6 hours) and plasma AUC (to 213 μg/h/mL), but limits its volume of distribution (2.0mL) and lowers the systemic exposure to free drug compared with the aqueous formulations. The reduced systemic exposure to VCR correlates with reduced toxicity, in particular neurotoxicity[32] and dermal toxicity; the latter is reduced because of the ability to retain the drug within the liposome at the site of drug administration.[37] However, the association of this formulation with increased anti-tumour activity, although evident, has not been adequately explained on the basis of its pharmacokinetic properties; in this regard, the accumulation of liposomes at tumour sites through the highly permeable vessels that characterise them, along with the slow release of VCR from the liposome, could account for the 10-fold increase of VCR in these areas.[32] Indeed, sphingosomal VCR demonstrates significantly improved activity against murine P388 leukaemia and human A431 squamous cell carcinoma tumours compared with both the free drug or VCR encapsulated in DSPC/chol liposomes.[32,36] Furthermore, sphingosomal VCR sensitises P glycoprotein (Pgp) 170-mediated VCR-resistant tumours, as evidenced by its activity in relevant tumour cell lines.[34]

A phase I clinical trial of liposomal VCR (in the form of DSPC/chol) in patients with refractory ma-

1 The use of trade names is for identification purposes only and does not imply endorsement.

lignancies, showed that increased doses of VCR can be administered when encapsulated in appropriate liposome formulations compared with conventional formulations, and established the dose of 2.0 mg/m^2 over 1-hour infusion, every 3 weeks, as a well tolerated dose for use in subsequent studies.[38] Pain in the form of myalgias and constipation, likely to be of autonomic aetiology, were the dose-limiting toxicities (observed at the dose level of 2.8 mg/m^2). The most frequent National Cancer Institute (NCI) Common Toxicity Criteria (CTC) grade 3–4 toxicities seen over all dose levels were constipation (12%), fatigue (8%), anaemia (8%) and alopecia (8%). Fevers and rigors likely related to the liposomal formulations were mild to moderate. Nausea and neuropathy were also reported; however, in general, neurotoxicity was less pronounced than that observed with free VCR. Liposomal VCR in the DSPC/chol formulation was also tested in a phase II trial of recurrent low- and intermediate-grade non-Hodgkin's lymphoma (NHL) patients. The formulation was well tolerated; nevertheless, neurotoxicity existed in a fraction of patients heavily exposed to prior neurotoxic agents.[39]

A phase II clinical trial of single-agent sphingosomal VCR given at a dose of 2.0 mg/m^2 every 2 weeks was conducted in patients with refractory/relapsed ALL.[40] Approximately 50% of the 16 patients receiving treatment had an initial CR duration of <1 year, 19% had not responded to standard induction chemotherapy and 50% had Ph+ disease. Sphingosomal VCR was the first salvage attempt in 69% of the patients. The overall objective response rate was 14% (one CR lasting 2 months and one partial response). These responses were achieved after the administration of two to three doses. Thirty-six percent of the patients had a transient reduction of marrow leukaemia infiltrate following two doses of therapy. Toxicity with a small number of doses was minimal and confined to mild peripheral neuropathy; however, long-term cumulative toxicity could not be evaluated because of VCR dose-intensity limitations.

Therefore, to achieve further dose intensity in the salvage ALL setting, a phase I–II clinical trial of sphingosomal VCR administered weekly with pulse dexamethasone was initiated: 30% of the patients were refractory to induction therapy and, overall, they had received a median of two prior salvage regimens. Of 14 patients who were evaluable for response, four (29%) attained CR (two at 1.5 mg/m^2, one at 1.825 mg/m^2 and one at 2.25 mg/m^2 of SV) and two (14%) showed haematological improvement (clearance of circulating leukaemia cells and transfusion independence of platelets). The rest of the patients either did not respond or their disease progressed. Peripheral neuropathy was seen in nearly all patients; however, it was generally mild.[41] These results suggested activity of sphingosomal VCR with dexamethasone in relapsed and refractory ALL, with allowance for dose intensification. The same VCR formulation was also substituted for conventional VCR as part of frontline therapy with cyclophosphamide, doxorubicin, VCR and prednisone (CHOP) +/− rituximab in patients with aggressive NHL, with good tolerability and 80% of evaluable patients achieving CR.[42] These results indicate that incorporation of sphingosomal VCR into frontline regimens for ALL could improve drug delivery and at the same time reduce toxicity.

1.3 Liposomal Annamycin

Doxorubicin is one of the most effective agents in the treatment of ALL. However, its use is often hindered by chronic cardiotoxicity and, importantly, by natural or acquired drug resistance; over-expression of the Pgp drug efflux pump occurs in a significant number of patients at disease relapse.[43] Annamycin (2′-iodo, 3′-hydroxy, 4′-epi, 4-dimethoxy doxorubicin) is a novel anthracycline antibiotic that is non-cross resistant and, additionally, has a high affinity for lipid membranes, which makes it ideal for liposome entrapment (as liposomal annamycin).[44] The drug incorporates four structural modifications from doxorubicin; substitution of the highly basic amino group at position 3′ of its sugar moiety by hydroxyl confers partial lack of cross-resistance without altering the interaction with topoisomerase II, and also reduces cardiotoxicity.[45,46] Iodination at position 2′ and demethoxylation at position 4′ of the aglycone portion both confer increased lipophilicity,[47] whereas axial orientation of the iodine atom is critical for preservation of high-biological activity.[44] Annamycin is carried in phospholipids (dimyristoyl phosphatidyl choline and dimyristoyl phosphatidyl glycerol).

Among many cell lines, *in vitro*, liposomal annamycin is as active as doxorubicin against doxorubicin-sensitive P388 leukaemia, but expresses a 50-fold higher potency against doxorubicin-resistant lines (P388/Dox), with calculated resistance indexes (concentration that produces 50% inhibition [IC_{50}] for resistant cells/IC_{50} for sensitive cells) with a value of 5 for liposomal annamycin versus 250 for doxorubicin. This increased potency is approximately the same for free and liposomal annamycin.[48] In contrast to doxorubicin, which is rapidly released from resistant cells, efflux of annamycin is similar in sensitive and resistant cells, thus suggesting that it is not mediated by Pgp. As a result, annamycin induces single- and double-DNA breaks in P388/Dox cells with a 300-fold higher potency than doxorubicin.[49] Liposomal annamycin displays reduced cardiotoxicity *in vitro* compared with doxorubicin.[50] *In vivo*, liposomal annamycin is more active in murine xenograft models against L-1210 leukaemia.[44]

The plasma concentration-time profiles for liposomal annamycin show a bi-exponential pattern with a rapid initial phase of distribution followed by a slower phase of elimination. Generally, maximum concentration (C_{max}) and AUC increase proportionally with dose. The drug's plasma terminal $t_{1/2}$ ranges from 1.1 to 2.5 hours.[51]

Based on its favourable preclinical evaluation, phase I clinical trials incorporating liposomal annamycin were conducted in patients with refractory/relapsed acute myeloid leukaemia (AML), ALL, chronic myeloid leukaemia in blastic phase (CML-BP)[52] and also in solid tumours,[51] mainly breast cancer.[53] In the solid tumour studies, the drug was well tolerated with no observed cardiotoxicity. Mild allergic reactions resulting from its formulation were experienced by a small number of patients. However, no objective responses were seen in solid tumours[51] and breast cancer in particular.[53]

In regard to haematological malignancies, 21 patients with relapsed/refractory disease including ALL (n = 3) and AML (n = 18), were enrolled on the dose-finding protocol using liposomal annamycin.[52] The drug was infused at a starting dose of 190 mg/m^2/day for 3 days, with escalation to 230, 280 and 350 mg/m^2/day for 3 days, by 1–2 hour IV infusion every 4–6 weeks. Therapy was generally well tolerated with no observed cardiotoxicity. The maximal tolerated dose (MTD) was determined at 280 mg/m^2/day for 3 days with grade 3/4 hepatotoxicity and mucositis observed at the higher dose level. Of 21 patients registered, one was enrolled but not treated, two had early deaths and 16 were unable to achieve CR. Two patients achieved CR, including one of three patients with ALL (at liposomal annamycin dosage of 350 mg/m^2/day), who had relapsed after allogeneic bone marrow transplant (BMT) and one patient with AML (at liposomal annamycin dosage of 280 mg/m^2/day), who had not responded to induction therapy with cyclophosphamide, cytarabine and topotecan. A second cycle of liposomal annamycin (280 mg/m^2/day) was started but the patient died as a result of progressive fungal infection, unrelated to study drug. The duration of response was 5 weeks. In general, the drug showed adequate myelosuppression. Of all the patients, 50% showed clearance of circulating blasts, and 43% showed clearance of bone marrow blasts. Therefore, liposomal annamycin appears to be well tolerated and has shown clinical activity in patients with acute leukaemia, and ALL in particular.

Consequently, a phase I/II clinical trial is currently being conducted to evaluate the safety and identify the MTD and anti-leukaemic activity of liposomal annamycin when given to patients ≥15 years with refractory or relapsed ALL. The starting dose is 190 mg/m^2/day over a 4-hour IV infusion for 3 days, in 21-day cycles, and with dose escalation up to 310 mg/m^2/day or to the MTD, whichever is achieved first. The study also aims to analyse markers of drug resistance such as multidrug resistance Pgp protein expression prior and post-therapy.

1.4 Pegylated Asparaginase

Asparagine is a crucial amino acid for protein, DNA and RNA synthesis, and appears to be a cell cycle-specific requirement for the G1 phase of cell division. Asparaginase, a bacterial enzyme, breaks down extracellular asparagine to aspartic acid and ammonia. Normal cells are capable of synthesising their own asparagines; however, ALL cells are unable to produce it and its depletion results in apoptotic cell death of the leukaemic cells.[54,55] The inclusion of L-asparaginase in the treatment regimen in paediatric ALL improved outcome.[56-60] A limitation in

the use of asparaginase is the development of hypersensitivity reactions and decreased plasma activity, which have been reported to be more severe in adults than in children.[61,62] Anti-asparaginase antibodies can reduce plasma asparaginase activity, leading to a rebound elevation of plasma asparagine and the possible development of drug resistance.[63-65] Pegaspargase (Oncospar®) is a modified form of native *Escherichia coli* asparaginase in which the enzyme is covalently linked to polyethylene glycol. The binding preserves the enzymatic function of the drug, decreases the immunogenicity of the protein, thus potentially reducing the risk of hypersensitivity reactions, and also increases its $t_{1/2}$.[66-68] Pegaspargase can be given to children with a history of allergic reactions to previous administration of *E. coli* asparaginase, and is indicated for patients who require asparaginase but have developed hypersensitivity to the native form.[69-71]

In adults with ALL, pegaspargase safety and efficacy data are limited. Recently, in a report by Wetzler et al.[72] for the Cancer and Leukemia Group B Study 9511, differences in overall survival (OS) and DFS between patients who did and did not achieve asparagine depletion were compared. The cut-off limit was defined by enzyme levels >0.03 units/mL plasma for 14 consecutive days after at least one of four planned pegylated asparaginase administrations. 102 patients were treated. Pegaspargase was administered at doses of 2000 IU/m^2, subcutaneously, capped at 3750U on day 5 of the five-drug induction course (cyclophosphamide, daunorubicin, VCR, prednisone and L-asparaginase) and day 15 of the early intensification course (2 months of treatment using cyclophosphamide, subcutaneous cytarabine, oral 6-mercaptopurine, VCR and L-asparaginase).[4] Pegaspargase was well tolerated, although hyperbilirubinaemia >3 mg/dL occurred in 54% of patients, hyperglycaemia >250 mg/dL in 40% and hypofibrinogenaemia <100 mg/dL in 30%. Samples were available from 85 eligible patients; 22 patients who did not achieve asparagine depletion had inferior OS ($p = 0.002$; hazard ratio [HR] = 2.37, 95% CI 1.38, 4.09) and DFS ($p = 0.012$; HR = 2.21, 95% CI 1.19, 4.13). After adjusting for age, performance status, leukocyte count and karyotype in a proportional hazards model, both the OS and DFS HR decreased to 1.8, concluding that effective asparagine depletion with pegaspargase is feasible as part of an intensive multi-agent therapeutic regimen in adult patients with ALL, and appears associated with improved outcomes.

Douer et al.[73] also reported on 25 ALL patients treated with a single-IV dose of pegaspargase 2000 IU/m^2 as part of a standard front-line induction regimen; the most common adverse effects were transaminitis (76%), hyperbilirubinaemia (72%) and hyperglycaemia (76%). The plasma fibrinogen level dropped below 100mg/dL in 15 patients (60%), with a median nadir level of 88 mg/dL. None of the patients had evidence of bleeding. In 16 patients (67%), the anti-thrombin III level dropped below 50% of baseline, and the median nadir level was 45% of normal control plasma. Out of 25 patients, 24 achieved a CR, including 23 patients who were in CR after phase I of induction. The one patient who did not achieve CR died of lung aspergillosis on day 27 of induction therapy. One patient with silent anti-pegaspargase antibodies achieved a CR. Thirteen patients relapsed, including ten with bone marrow relapse, one with isolated CNS relapse, one with relapse in bone and CNS, and one with relapse in bone marrow, CNS and testis. One patient died after an allogeneic BMT in first remission. Ten patients (40%) did not relapse and were alive at a median follow-up interval of 36 months. At 4 years, the OS and relapse-free survival were 31% and 40%, respectively.

2. Novel Antimicrotubule Agents

2.1 ABT-751

ABT-751 (figure 1) is a novel, oral methoxybenzene sulfonamide agent that inhibits microtubule polymerisation by binding tubulin, and therefore has a mechanism of action similar to VCR.

Microtubules are filamentous, tube-shaped protein polymers that are essential for the maintenance of cell shape, transport of vesicles, cell signalling and segregation of chromosomes during cell division by mitotic spindle formation.[74] They are composed of α-tubulin and β-tubulin heterodimers, which polymerise in a highly time- and space-regulated manner. Microtubules express functional di-

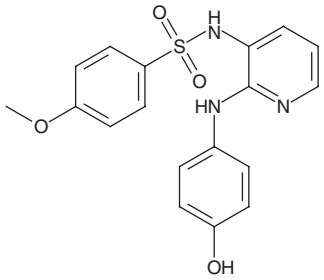

Fig. 1. Structure of ABT-751 (N-[2-[4-hydroxyphenyl)amino]-3-pyridinyl]-4-methoxybenzenesulfonamide).

versity, which is conveyed either by differences in tubulin structure and/or through the binding of various regulatory proteins to them. Microtubules constitute targets of a chemically diverse group of antimitotic agents with various tubulin-binding sites. Unlike drugs that bind the vinca domain (e.g. VCR, vinblastine, vinorelbine and cryptophycin 52), or the taxane domain (e.g. paclitaxel, docetaxel, epothilones), ABT-751 (as well as CI-980 [mivobulin] and combrestatins) binds to the colchicine domain on β-tubulin. The interference with normal microtubule dynamics leads to a block in the cell cycle at the G2/M phase, resulting in the induction of cellular apoptosis.[75-79]

Unlike vinca alkaloids and taxanes, ABT-751 is not a Pgp substrate. Furthermore, it inhibits the proliferation of a broad spectrum of human tumour-derived cell lines, including those that are doxorubicin- and paclitaxel-resistant due to the expression of Pgp.[80,81] This may be an important asset to this drug's efficacy, since the use of vinca alkaloids and taxanes is often limited as a result of the overexpression of proteins related to the multidrug resistance phenotype. Antiangiogenesis is another potential mechanism through which this drug may exert its anti-tumour effects; ABT-751 markedly reduced tumour blood flow in preclinical models, in doses where effects on normal vasculature were non-evident.[82,83] Several microtubule inhibitors are, reportedly, not devoid of similar antivascular actions, expressing tumour selectivity.[84,85]

ABT-751 is rapidly absorbed following oral administration, with time to C_{max} of ≈2 hours. From phase I studies in patients with solid tumours given ABT-751 for either 7 consecutive days during a 21-day cycle, or 21 consecutive days during a 28-day cycle, ABT-751 elimination was shown to follow linear pharmacokinetics, with both C_{max} and AUC increasing proportionally with increasing doses. The $t_{1/2}$ of the drug is ≈5 hours and it accumulates minimally after daily or twice-daily administration. It binds to plasma proteins extensively (mean 98.6%).[82,86-89]

ABT-751 is active against a variety of human tumour-derived syngeneic and xenograft animal models, including colon,[90] gastric, sarcoma and breast cancer, as well as Lewis lung carcinoma.[91] ABT-751 potently inhibits intraperitoneally inoculated murine P388 leukaemia at submicromolar IC_{50} values (IC_{50} = 0.16–0.86μM).[92-94]

ABT-751 has been administered to patients with solid tumours in several different dosage schedules and in the setting of phase I and II trials.[95] Phase I studies were conducted with oral ABT-751 administered to patients in a single-dose (80–480 mg/m^2) [n = 16], 5-day repeated dose (30–240 mg/m^2) [n = 41] or 7-day repeated dose every 3 weeks (200–300 mg/day or 125–175mg twice daily). MTDs were determined as 320 mg/m^2 for the single-dose study, 200 mg/m^2 for the 5-day repeated-dose study, 250 mg/day for the 7-day dose (once daily) and 175 mg/day for the 7-day dose (twice daily). Peripheral neuropathy and ileus were dose-limiting toxicities in all three studies. Overall, haematological toxicities were mild, but not dose dependent. Gastrointestinal toxicities were dose dependent, but not severe. Anti-tumour activity in the form of tumour regression, or reduction in tumour markers, was observed in a small number of patients.[86]

Subsequently, studies in preclinical models revealed that enhanced anti-tumour efficacy without added toxicity could be achieved with long-term administration on a 21–28 day schedule. A consecutive 21-day on, 7-day off in a 28-day cycle schedule was explored in a phase I trial of chemotherapy-resistant solid tumours, mainly colorectal cancer. Escalating flat doses of 25–250mg once daily (n = 26), or 25–100mg twice daily (n = 17) were given. Although this study is ongoing, ileus NCI CTC grade 4), is the dose-limiting toxicity observed in one-eighth of patients at 200mg and one-third of patients at 250mg, respectively. Hyperbilirubinaemia (NCI CTC grade 3) was seen in one-eighth

of patients and hyponatraemia (NCI CTC grade 3) in one-eighth of patients, both at the 100mg twice-daily dosage schedule. No noteworthy myelosuppression has been seen. Moderate constipation, anorexia and abdominal pain were also seen in a very small number of patients. One minor response (tumour marker reduction) has been observed in a patient with colorectal cancer.[96]

Since the above schema was shown to have a favourable toxicity profile, it was introduced in a phase I clinical trial in patients with relapsed-acute or treatment-refractory leukaemia, or advanced myelodysplastic syndrome (MDS). Thirty-two patients were treated: nine with 100 (n = 3), 125 (n = 3) or 150 mg/m^2 (n = 3) of ABT-751 given orally once daily for 7 days every 3 weeks; and 23 with 75 (n = 3), 100 (n = 3), 125 (n = 5), 150 (n = 5), 175 (n = 3) or 200 mg/m^2 (n = 4) of ABT-751 given orally once daily on a 21-day on, 7-day off in a 28-day cycle schedule. The former schema was substituted for the latter after the results of the relevant phase I study in solid tumours became available. Twenty-nine patients (91%) had AML or MDS; three (9%) had ALL. The majority of the patients were heavily pretreated. Two (67%) patients with ALL had a normal karyotype. Dose-limiting toxicity consisted of ileus in one patient at 200 mg/m^2, with a subsequent patient developing grade 2 constipation at the same dose level. The study indicated that the recommended phase II dose for patients with haematological malignancies is 175 mg/m^2 daily orally for 21 days, every 28 days. Non-haematological toxicities in the 7-day administration schedule were mild with a low prevalence and included vomiting, diarrhoea, constipation and hyperbilirubinaemia. Grade 1 or 2 adverse events were documented at all dose levels for patients receiving the 21 of 28-day regimen. No consistent or significant increment in the frequency of NCI CTC grade 2 toxicities was observed as the dose of ABT-751 was escalated. The most frequent toxicities were gastrointestinal (i.e. nausea and or vomiting [56%], constipation [43%], diarrhoea [39%], anorexia [26%], abdominal pain [13%], mucositis [13%], and elevations of transaminases, bilirubin and/or alkaline phosphatase [13%]). Neurological symptoms included peripheral neuropathy (17%), dizziness (4%) and somnolence (4%). Fatigue was observed in 17% of patients, bone pain in 13% and weight loss in 13%. One patient experienced a grade 3 ileus/small bowel obstruction during the first cycle of treatment at the 200 mg/m^2 dose, which resolved with conservative management and discontinuation of ABT-751. Two patients, with diagnoses of AML and ALL, treated with ABT-751 at a dose of 175 mg/m^2 for 21 days every 28 days, had a reduction in the percentage of peripheral blood blasts from 43% to 5% and from 94% to 0%, respectively. In the first patient, the blasts increased to 33% with the discontinuation of ABT-751 during the 7-day drug-free period but disappeared completely on re-initiation of the drug. This patient subsequently decided to withdraw from further therapy. The second patient was taken off the study because of disease progression in the bone marrow.[97]

In essence, ABT-751 is a well tolerated regimen for patients with ALL and haematological malignancies in general, allowing for the administration of higher doses than those used in patients with solid tumours. All three patients (9%) with ALL in this study had received prior VCR therapy, two of whom had grade 1 or 2 peripheral paresthesias at baseline, without any worsening of these symptoms on study.

The impact of ABT-751 on ALL can not be accurately assessed thus far because of the very low number of patients treated; however, the incidence of a significant mid-cycle reduction of bone marrow leukaemia infiltrate (one-third of patients) may indicate that ABT-751 warrants further investigation in this subset of patients. Altered expression of β-tubulin isotypes (especially β3 to which ABT-751 binds with high affinity) in P388 leukaemia subclones carrying relevant mutations, may render them resistant to ABT-751.[92] In this regard, several single-nucleotide polymorphisms (including the recently described Ala185Thr in exon 4 of β-tubulin gene) have been identified, yet their significance has not been elucidated.[97-99]

The number of circulating endothelial cells (CECs) reflects angiogenesis and bone marrow activity in leukaemia, and may constitute a leukaemic biomarker.[100,101] The proportion of CECs undergoing apoptosis reportedly did not increase following administration of ABT-751 in leukaemia, casting doubt upon previous commentaries which attribute

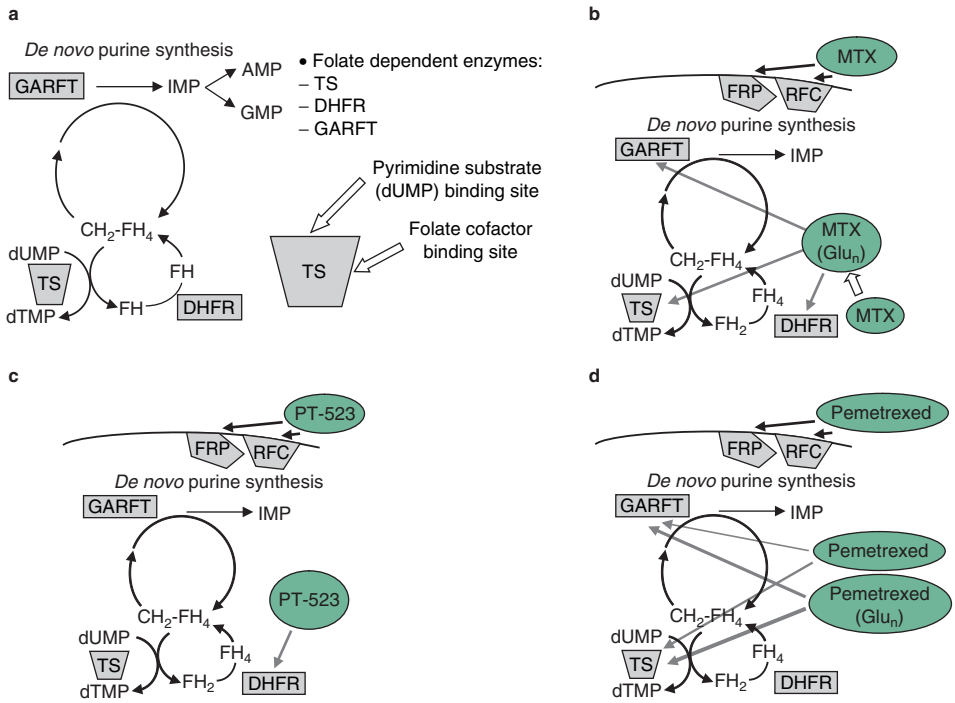

Fig. 2. The role of folates in DNA synthesis (**a**), and mechanisms of action of methotrexate (MTX) (**b**), talotrexin (PT-523) [**c**] and pemetrexed (**d**). **AMP** = adenosine monophosphate; **DHFR** = dihydrofolate reductase; **dTMP** = deoxythymidine monophosphate; **dUMP =** deoxyuridine monophosphate; **FRP** = folate receptor protein; **GARFT** = glycinamide ribonucleotide formyltransferase; **Glu**$_n$ = polyglutamate; **GMP** = guanosine monophosphate; **IMP** = inosine monophosphate; **RFC** = reduced folate carrier; **TS** = thymidylate synthetase.

anti-angiogenesis as a candidate mechanism of action of ABT-751.[102]

3. Antifolates

MTX, an inhibitor of dihydrofolate reductase (DHFR), is active in ALL and is traditionally included in induction and/or consolidation programmes. MTX enters the cell by an active transport process and in the cell it becomes polyglutamated, a process that favours its intracellular retention. The intracellular formation of polyglutamates results in inhibition of DHFR, the enzyme responsible for conversion of folate and dihydrofolate to tetrahydrofolate. The latter compound is involved in the conversion of deoxycytidine monophosphate to thymidine monophosphate by thymidylate synthetase for biosynthesis of purines required for incorporation into DNA. Although some newer generation antifolates (e.g. trimetrexate) have been evaluated in adult and paediatric ALL,[103,104] others, such as pemetrexed and talotrexin, have only very recently been entered in clinical trials (figure 2, table I).

3.1 Pemetrexed

Pemetrexed disodium (LY231514, Alimta®) is a novel antifolate antimetabolite which, in addition to DHFR, inhibits several key folate-requiring enzymes, including thymidylate synthetase (TS), glycinamide ribonucleotide formyltransferase and aminoimidazole carboxamide formyl transferase.[105-107] Inhibition of these enzymes results in the depletion of active folate and nucleotide pools and, therefore, impedes DNA and RNA synthesis. Consequently, the cell cycle is arrested in the G1/S phase[105,108] (figure 2). The drug is taken up by the cell membrane, preferentially by the reduced folate carrier (RFC),[109] and once inside the cell it undergoes polyglutamation, resulting it its intracellular retention in a manner similar to natural folates.[110] It is worth mentioning that resistance to antifolates

Table I. Antifolates: mechanisms of action and resistance

Agent	Mechanism(s) of action	Mechanism(s) of resistance
Methotrexate	Enters cell by active transport	Defective membrane transport carrier system
	Polyglutamates of MTX and dihydrofolate inhibit purine and thymidylate biosynthesis	Decreased level FPGS
	Inhibition of DHFR; depletion of reduced folates	Decreased affinity for DHFR by isoenzymes, *DHFR* gene amplification, increased DHFR protein
Trimetrexate	Enters cell by passive diffusion	
	No polyglutamation	
	Inhibition of DHFR; depletion of reduced folates	Decreased affinity for DHFR by isoenzymes, *DHFR* gene amplification, increased DHFR protein
Pemetrexed	Enters cell by active transport	Defective membrane transport carrier system
	Polyglutamates of MTX and dihydrofolate inhibit purine and thymidylate biosynthesis	Decreased level FPGS
	Inhibition of DHFR, TS, GARFT, AICARFT; depletion of reduced folates	Decreased affinity for enzymes, enzyme gene amplification, increased DHFR protein
Talotrexin	Enters cell by active transport more efficiently than MTX	Defective membrane transport carrier system
	No polyglutamation	
	Inhibition of DHFR, more potently than MTX; depletion of reduced folates	Decreased affinity for DHFR by isoenzymes, *DHFR* gene amplification, Increased DHFR protein

AICARFT = aminoimidazole carboxamide formyl transferase**; DHFR** = dihydrofolate reductase; **FPGS** = folylpolyglutamyl synthetase; **GARFT** = glycinamide ribonucleotide formyltransferase; **MTX** = methotrexate; **TS** = thymidylate synthetase.

was shown to develop *in vitro* by several mechanisms, including alterations in drug uptake (decreased RFC expression)[111] or in the activity of target enzymes.[112]

Pemetrexed has been extensively evaluated in clinical trials for patients with solid tumours, but not in those with haematological malignancies. To date, >12 000 patients with solid tumours have received pemetrexed through various phase I, II and III clinical trials. This agent has shown broad-spectrum activity in multiple-tumour types when given as a single agent once every 21 days at 500 or 600 mg/m^2. Phase II studies have demonstrated activity in breast,[113] colorectal,[114] gastric, non-small cell lung,[115-119] head and neck[120] and pancreatic cancers[121], and in malignant pleural mesothelioma.[122-124] The pemetrexed plus cisplatin combination demonstrated a 30% improvement in median survival time in mesothelioma, as compared with cisplatin alone (12.1 vs 9.3 months; log-rank p = 0.02).[125] The drug was approved by the US FDA in combination with cisplatin for the treatment of unresectable malignant pleural mesothelioma and as single agent in second-line treatment of non-small cell lung cancer.

Haematological toxicity has been identified as the most prevalent and serious toxicity after administration of single-agent pemetrexed, with absolute neutrophil and platelet nadirs decreasing with increase of systemic exposure (represented by increases in AUC). The effect of pemetrexed on neutropenic response is not necessarily cumulative after multiple treatment cycles.[126] Gastrointestinal toxicity (nausea, mucositis and diarrhoea) [seen in 10% of the patients], skin rash (seen in 5% of patients), fatigue and transient liver transaminase elevations, are the commonest non-haematological toxicities. Dose reductions (by 25%) throughout therapy are feasible and may be implemented in the event of severe (NCI CTC grade 3–4) non-haematological toxicities (other than transaminase elevations) [reduction by 25%] or mucositis (reduction by 50%). Severe neurotoxicity may necessitate drug discontinuation.

Pemetrexed is eliminated primarily by renal excretion, mostly as unchanged drug, with an elimination $t_{1/2}$ from plasma of 3.5 hours. Its AUC correlates with its systemic clearance and its safety, efficacy and pharmacokinetics have not been systematically studied in a sufficient number of patients with creatinine clearance <45 mL/min.[126] Compared with 5-

formyltetrahydrofolate (also known as leucovorin or folinic acid), folic acid has a less prominent effect on the action of pemetrexed and causes only 4-fold increases to its IC_{50} (vs 370-fold increases by leucovorin). Thus, folic acid and vitamin B12 (cyanocobalamin) supplementation are considered appropriate for patients receiving pemetrexed in order to alleviate toxicity. The current recommended regimen of single-agent pemetrexed is 500 mg/m^2 on day 1 of a 21-day cycle, with daily oral folic acid (350–1000μg) given 7 days preceding, during and 21-days post-administration, vitamin B12 (1000μg intamuscularly) once every 9 weeks starting the week preceding the first dose of the drug and, unless clinically contraindicated, dexamethasone 4mg (or equivalent) per day for 3 days, starting 1 day before each pemetrexed dose to prevent skin rash. Even after vitamin supplementation was introduced and higher doses were tested, it was shown that higher doses than recommended did not increase anti-tumour activity and were associated with marginally higher toxicity.

Pemetrexed exhibits highly cytotoxic *in vitro* activity against the CCRF-CEM human T-cell lymphoblastic leukaemia cell line. With myelosuppression being its dose-limiting toxicity, it displays a favourable profile in drug development for the treatment of leukaemia. As a result of such a safety profile, dose increases beyond the conventional ones (500 mg/m^2) used in solid tumours are probably feasible. A phase I/II study to determine the MTD, pharmacokinetics and to assess response rates with pemetrexed therapy in patients with refractory/relapsed ALL among other haematological malignancies, is currently being conducted and results are awaited. Pemetrexed cellular uptake and activation, levels of targeted enzymes and downstream molecular events will also be assessed by correlative studies.

3.2 Talotrexin

Talotrexin (PT-523, Talvesta™), is another antifolate antimetabolite that inhibits the enzyme DHFR (figure 2). Owing to its unique structural motifs, the drug combines characteristics of both the classical antifolates (e.g. MTX, aminopterin), such as water-solubility, and the nonclassical ones (e.g. trimetrexate) that do not contain a glutamic acid side chain and thus are incapable of being converted to noneffluxing polyglutamates once they enter a cell. Although polyglutamation is an important mechanism for drug retention, it may render malignant cells with a low efficiency to form such metabolites, resistant to classical antifolates.[127] Moreover, since normal proliferative tissues are more efficient in polyglutamating, haematopoietic and gastrointestinal toxicities take precedence. This type of resistance is circumvented by talotrexin. Talotrexin enters cells, at least in part, via the RFC transport system, and ≈10-fold more efficiently than MTX.[128] However, this mechanism of uptake may also constitute a means of resistance, as has been described in leukaemia cell lines expressing inactivating mutations or allele loss of the RFC system.[129] It binds to DHFR much more tightly than MTX, with an inhibitory constant (Ki) of 0.35 pmol/L, which is 15-fold lower than MTX. Its high affinity for DHFR suggests that certain elements of its structure cause dissociation from the active site to be very slow in comparison to MTX and other classic DHFR inhibitors.[130,131] As a result, *in vitro* talotrexin inhibits *de novo* pyrimidine and purine synthesis more potently than either MTX or trimetrexate.[132] Moreover, unlike trimetrexate, which is more lipophilic, cells that over-express Pgp (e.g. L1210/MDR), are not resistant to talotrexin.[131]

As previously indicated, the concentration of folic acid can modulate the sensitivity of the cells to antifolates; however, in murine L1210 leukaemia cells, the IC_{50} of talotrexin only changes by a factor of 2 as the concentration of the reduced folate in the medium increases 100-fold, whereas the difference is 6-fold for MTX and >10-fold for other polyglutamable antifolates. This suggests that talotrexin may be less dependent upon folate homeostasis than MTX and drugs that belong to the same group with it.[133]

In vitro, and among other cell lines, talotrexin inhibits the proliferation of murine B-cell leukaemia (L1210 cells) and human T-cell leukaemia (CCRF-CEM cells) with excellent potency even in cells with a clinically relevant (i.e. 10- to 20-fold) level of resistance to MTX and other polyglutamable compounds. The inhibitory effect of talotrexin on these cell lines increases with prolonged duration of exposure, suggesting that the drug might be a schedule-

dependent cytotoxic agent. This is consistent with the inability of this drug to undergo polyglutamation.[134,135] *In vivo*, talotrexin is more effective against L1210 leukaemia compared with equiactive MTX doses.[136]

A thorough pharmacokinetic analysis of talotrexin in humans has not been completed; however, preliminary data demonstrate an increase of both C_{max} and AUC with increasing drug doses, but less than dose-proportionally.[137] In animal species, and within the range of doses evaluated, the drug appeared to exhibit apparent linear pharmacokinetics with a very rapid initial disposition phase.

Talotrexin only very recently entered the clinical trial arena and was evaluated in a phase I study including patients with haematological malignancies.[137] Patients with relapsed or refractory leukaemia or MDS were enrolled in successive cohorts to receive talotrexin based on body surface area (mg/m^2) as a 5–10 minute infusion with pharmacokinetic sampling on days 1–5, and observation until day 21. All patients received folic acid and B12 vitamin supplementation prior to initial administration of talotrexin.

Twenty-eight patients have been enrolled to date and have received one complete cycle (five doses) of talotrexin. Dose-limiting toxicities were observed at 0.8 mg/m^2 (4 mg/m^2 per cycle) and consisted of two cases of NCI CTC grade 3 oral mucositis. The every 3-week MTD was 0.6 mg/m^2. Four patients were enrolled in an expanded MTD cohort, and adverse events consisted of mucositis, gingival inflammation, pneumonia, hypokalaemia, hypomagnesaemia, fever (neutropenic/non-neutropenic), fatigue, nausea, alopecia, bruising and cough. Three major responses were observed, including one patient each with ALL, AML and CML-BP. A phase II study of talotrexin in refractory ALL is now ongoing and results are awaited.[137]

4. Nucleoside Analogues

4.1 Nelarabine

Nelarabine, which recently received accelerated approval by the FDA for the treatment of patients with relapsed or refractory T-cell ALL/lymphoblastic lymphoma, is a pro drug of the deoxyguanosine analogue arabisubnofuranosylguanine (ara-G), a compound originally synthesised in 1964[138] (figure 3). As reviewed by Gandhi et al.,[139] clinical interest in guanine nucleoside analogs was activated with the discovery that genetic deficiency of purine nucleoside phosphorylase results in a profound T-cell lymphopenia. Pharmacology studies demonstrated that cytotoxicity was associated with elevated plasma deoxyguanosine and a pronounced intracellular accumulation of deoxyguanosine triphosphate (dGTP). Models of this metabolic disease demonstrated that immature T lymphocytes and T-lymphoblastoid cells were selectively sensitive to treatment with deoxyguanosine, whereas lymphocytes of B-cell lineage did not accumulate high levels of dGTP and were much less sensitive to deoxyguanosine.[139]

Nelarabine requires demethylation by adenosine deaminase to form its active compound (figure 3). Intracellular deoxyguanosine kinase and deoxycytidine kinase phosphorylate ara-G sequentially to form ara-GTP. Phosphorylation of ara-G to ara-GTP is rapid, and intracellular exposure to ara-GTP is much higher than the exposure to intracellular ara-G or nelarabine. After phosphorylation, ara-GTP substitutes for GTP in numerous biological processes, including the replication of DNA. This substitution leads to inhibition of DNA synthesis resulting in cell

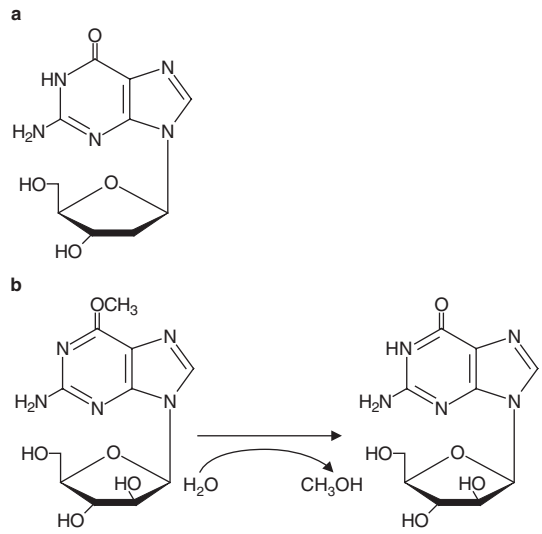

Fig. 3. Structure of deoxyguanosine (**a**) and conversion of nelarabine to arabisubnofuranosylguanine (**b**).[139]

death. This process is lethal to malignant T cells, as it is to other rapidly replicating cells.[140-142]

T-cell lymphoblastic leukaemia/lymphoma, are the disease entities in which nelarabine has been used to date with success. In two phase II trials, in paediatric and adult patients, nelarabine was administered at 650 mg/m^2/day for 5 days and 1.5gm/m^2/day on days 1, 3, 5 of 22-day cycles, respectively. Overall, response to nelarabine was observed in 36% of 39 paediatric patients, CR in five (13%) and CR with incomplete haematological or bone marrow recovery in 9 (23%). Adults showed an OS of 39% out of 28 ALL patients, CR in five (18%), and CR with incomplete haematological or bone marrow recovery in six (21%). Neurological toxicity was dose limiting for both paediatric and adult patients, consisting of headache, peripheral neuropathy, somnolence, ataxia and seizures. Other severe toxicities included laboratory abnormalities in paediatric patients and gastrointestinal and pulmonary toxicities in adults.[142]

Analogous results were recently reported in a cohort of 26 adult patients with refractory/relapsed T-cell ALL and 13 with T-cell lymphoblastic lymphoma, at the same dosage regimens of the drug.[143] Nelarabine induced a 31% CR and a 41% OS rate. Myelosupression was the most significant toxicity, experienced by ≈30% of patients. A CNS complication (depressed level of consciousness) was the only serious (grade 4) adverse event observed. The median DFS and OS were both 20 months, and the 1-year OS was 28%.

5. Novel BCR-ABL Inhibitors

Imatinib mesylate (imatinib, Glivec®, Gleevec®) has had a profound impact on the management of Ph+ ALL, which accounts for ≈25% of all adult ALL. However, imatinib is not sufficient to eradicate advanced (i.e. refractory or relapsed) ALL because of the development of resistance.[144] Therefore, its incorporation to therapeutic regimens along with cytotoxic agents is usually warranted. It is now known that the combination of intensive chemotherapy with imatinib as frontline therapy can attain complete haematological responses of up to 90%, increase the molecular remissions by 50% and reduce treatment-related mortality in Ph+ ALL patients compared with chemotherapy alone.[145,146]

True resistance to imatinib is sometimes associated with ABL kinase domain mutations or overexpression of BCR-ABL itself. The rate of such mutations is higher in Ph+ ALL than in CML, and especially at disease relapse and in elderly patients. Improved understanding of the interaction between imatinib and its target led to the design of nilotinib (AMN107, Tasigna®), whereas dasatinib (Sprycel™) was developed in a search for Src kinase inhibitors. Both agents are expected to alter the therapy of ALL, particularly for patients with imatinib-resistant disease.

5.1 Nilotinib

The imatinib derivative nilotinib is an aminopyrimidine highly selective ABL kinase inhibitor with 20- to 50-fold greater potency than imatinib. *In vitro*, cell lines carrying the BCR-ABL mutation T315I were the only resistant cell lines in 32 of 33 tested that demonstrated nilotinib insensitivity.[147] Other translocation targets of nilotinib include FIP1L1-PDGFR-α, TEL-PDGFR-β and D816V-mutated KIT, potentially extending its spectrum of activity to idiopathic hypereosinophilic syndrome, chronic myelomonocytic leukaemia and systemic mastocytosis. Following manipulation of the N-methylpiperazine group of imatinib to produce nilotinib, crystalography studies reveal that nilotinib fits more tightly into BCR-ABL's adenosine triphosphate binding pocket than imatinib, thereby producing a 3- to 7-fold more potent inhibitory effect in imatinib-resistant cell lines.

In vitro evidence also suggests that kinase domain mutations are rarer with nilotinib treatment and respond to higher doses of the drug (with the exception of the BCR-ABL mutation T315I).[148] Nilotinib treatment of mouse models with imatinib-resistant BCR-ABL transformed cell lines resulted in prolonged survival for these animals. These data led to the conduct of a phase I study of nilotinib in patients with imatinib-resistant CML and Ph+ ALL.[149] In this study, 119 patients were enrolled and nilotinib was administered orally in escalating doses from 50mg to 1200mg daily. The toxic low dose for nilotinib was set at 600mg daily with myelosuppression (grade 3–4 thrombocytopenia and neutropenia in 20% and 13% of patients, respectively), skin rashes and transient hyperbilirubinaemia

occurring with higher doses. Of 17 patients in chronic phase, nine (53%) obtained a cytogenetic response (complete cytogenic remission [CCyR] in six cases) following a median duration of therapy of 4.9 months, 11 of 12 patients (92%) with active disease had a haematological remission. In patients with accelerated disease (n = 51), 74% (n = 38) obtained a haematological response and 55% (n = 31) had a cytogenetic response (major in 15 cases). Of those with blast crisis (n = 33), haematological and cytogenetic responses were observed in 39% (n = 13) and 27% (n = 9) of patients, respectively. In patients with haematological relapse of Ph+ ALL, one of ten patients had a partial haematological response. The *in vitro* experience with the T315I mutation was confirmed in this trial, with patients expressing this mutation showing resistance to nilotinib. Equivalent activity was seen in BCR-ABL mutated and unmutated cases. Large phase II studies in imatinib-resistant or intolerant ALL patients at doses of nilotinib 400mg twice daily are now enrolling patients.[150]

5.2 Dasatinib

The Src family of kinases consists of a group of nine non-receptor intracellular tyrosine kinases (Src, Fyn, Lyn, Hck, Yes, Yrk, Fgr, Blk and Lck) that regulate cell growth and survival. The Src kinases are critical players in the pathogenesis of CML. Multiple BCR-ABL domains interact with both Hck and Lyn. The BCR-ABL-Hck-STAT5 pathway produces myeloid transformation *in vitro* and plays an important role in Ph+ leukaemias.[151] Over-expression of the Src kinases has been implicated in BCR-ABL driven disease progression and imatinib resistance. *In vitro* models suggest an important role for Lyn, Hck and Fgr expression in lymphoid blast crisis,[152] and other Src kinases may be active in leukaemia according to the tissue-specific expression of various Src family kinase members. Activated ABL has close sequence homology with the Src kinases. It is not surprising then that dasatinib (BMS-354825), initially conceived as a Src kinase inhibitor, is in fact, a multi-targeted ABL/Src inhibitor and inactivates BCR-ABL, EPHA2, KIT, PDGFR as well as the Src kinases. Similar to nilotinib,[147] *in vitro* studies have shown activity in all BCR-ABL mutated cell lines except for T315I cases; additionally, a survival advantage for dasatinib treated, imatinib-resistant BCR-ABL mice has also been demonstrated.[152]

A multicentre phase II analysis of dasatinib entitled 'START' (Src/ABL Tyrosine kinase inhibition Activity: Research Trials of dasatinib) consisted of five separate trials: one randomised trial comparing dasatinib versus high-dose imatinib in resistant/intolerant CML (all stages)[153] and four single-arm studies of dasatinib in patients with imatinib-resistant/intolerant CML and Ph+ ALL.[154-157] In lymphoid blast crisis, major cytogenetic remissions were seen in 44% (CCyR 38%) of patients. Patients with Ph+ ALL (n = 46) had equivalent response rates with major cytogenetic remissions in 46% (CCyR 44%). Non-haematological toxicities included diarrhoea, headache, pleural effusions and fluid retention. NCI CTC grade 3–4 neutopenia or thrombocytopenia was seen in 47% of patients. The long-term consequences of Src family kinase inhibition with dasatinib are unknown. *In vitro* developmental studies on mice have produced impaired immune function, memory disturbance and osteopetrosis following selective Src family kinase inhibition; however, the impact of this on fully developed organ systems remains to be established.

5.3 Other Multi-Targeted Kinase Inhibitors

Of the multi-targeted kinase inhibitors in clinical development, most data are currently available on dasatinib, which is now approved for the therapy of Ph+ ALL. Phase I studies of other Src/ABL small molecule inhibitors, bosutinib (SKI-606) and INNO406 (NS-187) are underway.

Aurora kinases are a family of serine/threonine kinases important in cell-cycle regulation and are over-expressed in a variety of human malignancies. The small molecule MK0457 (VX-680) produces effective inhibition of Aurora kinases in imatinib-resistant CML *in vitro*, and efficacy has been shown in cell lines harbouring the BCR-ABL T315I mutation. Importantly, MK0457 is the first drug to display activity against this mutation in the clinical arena, as response to this agent has been reported in patients with T315I-mutated Ph+ ALL.[158] Numerous different approaches to inhibition of BCR-ABL and/or its substrates are under investigation.

6. Conclusion

This review does not aim to cite a complete catalogue of the novel compounds that could potentially be applied in adult ALL therapy; rather, it focuses on some of the newer agents and formulations, whose clinical evaluation has rendered promising results (e.g. sphingosomal VCR, pegaspargase, nelarabine, BCR-ABL inhibitors) or that have only recently entered the clinical trial arena, for which very limited, if any, bibliographic information currently exists (e.g. liposomal annamycin, novel antifolates, ABT-751). In regards to the latter, an attempt is made to justify, based on preclinical data, their incorporation into clinical trials. However, as a result of their early developmental stage, it is still unclear as to whether their distinct mechanisms of action, or even their ability to circumvent resistance *in vitro*, will be translated into significant antileukaemic efficacy in patients with ALL. At the same time, other compounds (e.g. signal transduction pathway [i.e. 'notch'] inhibitors, monoclonal antibodies) may prove conceptually attractive.

Although with the modern therapeutic induction schemas, the majority of adult ALL patients manage to attain CR initially, a relatively small percentage of them retain this remission in the long-term. In addition, because the overall prognosis remains poor, especially in relapsed or refractory adult ALL, a palliative approach to the management of multiply relapsed or refractory ALL should be supplemented by enrolment into clinical trials incorporating new agents to promote drug discovery. Targeted inhibition of leukaemogenic pathways has made a profound impact on the management of ALL (e.g. BCR-ABL inhibitors in Ph+ ALL); however, it has been hindered by resistance over time to these agents. In this regard, combination therapy programmes of established chemotherapy agents with new agents, based on rational approaches, should be pursued. New technologies, such as DNA microarray analysis with gene expression profiling, may guide such therapeutic choices in future studies.[159]

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