

## Chapter 2

# Identifying Targets for New Therapies in Children with Acute Lymphoblastic Leukemia

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### Summary

For those of us who look after children with acute lymphoblastic leukemia (ALL), these are heady times. Cure rates on current therapeutic regimens are now approaching 90% [1, 2]. Therapy is almost entirely chemotherapy-based with very few patients now receiving irradiation [3]. Why then in this group of patients should we be looking for new agents? The obvious one is that we are reaching the limits of what can be achieved with combination chemotherapy [4]. In a sense we have been lucky. Almost all of the earliest chemotherapeutic agents proved effective in childhood ALL. Children tolerate combination chemotherapy better than adults. This has allowed us to gradually intensify therapy in all groups and in particular those at a higher risk of relapse. This risk-stratified approach to intensification has proven to be highly effective [5–11]. One problem we now face is the high cost of cure. Treatment-related mortality and morbidity [12] is almost balancing out the relative risk of relapse. Allogeneic stem cell transplant (allo-SCT), the ultimate in treatment intensity, cannot cure patients unless disease burden is first reduced using chemotherapy [13, 14]. Thus, intensification of therapy is unlikely to improve outcome any further. We therefore need new drugs not only to cure those currently failed by therapy but also to decrease the morbidity of current treatment. At present most protocols use ten or more drugs over a period of 2–3 years to treat children with ALL. The cost of treatment and supportive care is prohibitive for countries with restricted resources. This includes the most heavily populated parts of the world. Thus, the remarkable success rates seen in developed countries are yet to be translated globally [15]. To provide a solution for all children with ALL we need shorter,

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cheaper therapeutic strategies. Finally, childhood ALL is a paradigm for successful cancer therapy. In terms of modern biology, it is one of the most heavily investigated. In a sense, having resolved the therapeutic dilemma we now have the luxury of dissecting out the mechanisms of cure and resistance. It is likely that the biological mechanisms that regulate the variations in the therapeutic response and side effects are common to more than one tumour type. Thus, the mechanisms identified are likely to have wider application in the treatment of cancer.

## 2.1 Understanding Disease Biology

In the following sections, we pose key questions, the solutions to which we believe are fundamental in advancing and refining therapy in childhood ALL.

## 2.2 Can We Further Optimise Current Therapy?

Biologically, childhood ALL is a heterogeneous disease. Cytogenetic analysis demonstrates this and outcome clearly varies by genetic subtype. However, even within a cytogenetic subset, there is disparity. A small proportion of those with hyperdiploidy relapse. Similarly, a small proportion of those with Philadelphia chromosome positive (Ph+) ALL respond well to chemotherapy. As hyperdiploidy is more common, relapse in this group poses a bigger clinical problem. Biological heterogeneity is also reflected in the therapy used. Drugs used predominantly affect nucleic acid integrity (intercalating agents, epipodophyllotoxins), synthesis (anti-metabolites), replication (mitotic spindle poisons) and transcription (steroids). Notable in this armamentarium is the drug L-asparaginase, which exerts a unique cytotoxic effect specific to this cancer and is a pivotal drug in the treatment of childhood ALL. The most sensitive predictor of outcome has proven to be the early response to therapy, measured either by a decrease in circulating blasts, percentage residual blasts in the marrow or molecular level of disease during initial therapy. Thus, the heterogeneity of disease has serendipitously been tackled by the use of multi-targeted therapy effective against the most frequent subtypes of childhood ALL. This accords with the Goldie–Coldman hypothesis [16] which avers that drug resistant clones are less likely to evolve in tumours treated with the most effective combination chemotherapy. However, while this sweeping approach may stochastically benefit the majority, a proportion of patients will necessarily be over treated and some will not receive appropriate therapy. Further fine-tuning is still possible with current chemotherapeutic agents. For example, there is evidence to suggest that patients with *ETV6-RUNX1* ALL are more sensitive to asparaginase [17] and that overexpression of the folate reductase carrier gene through duplication of chromosome 21 renders patients with hyperdiploid ALL more sensitive to methotrexate [18]. Thus, protocols could be adapted to increase exposure of the specific drug in each cytogenetic category [19]. The problem we face is that we are still unsure about the precise

mechanisms of actions of drugs and the consequences of their interactions. This limits our ability to predict recurrences. A better understanding of the biological processes is now ushering in an era of individualised therapy. An exemplar of this is the ABL tyrosine kinase inhibitors in Ph+ ALL [20].

### 2.3 What Are the Origins of Relapse?

Relapsed ALL is broadly risk-stratified by the duration of first remission. Those who relapse early are often incurable, even with allo-SCT. In contrast those who relapse late, off therapy, have survival rates of over 70% with conventional chemotherapy [21–23]. Genome-wide analysis has recently shown that in almost 90% of cases, disease recurrence is due to a sub-clone present at original diagnosis [24]. This observation is supported by results of recent xenotransplantation experiments performed in more permissive immunodeficient mice recipients. In these studies, lymphoblast populations designated mature by immunophenotypic criteria also appear to possess stem cell properties, suggesting that “stemness” in ALL is more widely prevalent than previously recognised [25]. Gene expression profiling (GEP) has also been used to investigate relapsed disease [26–28]. GEP analyses suggest that transcriptional signatures differ between diagnostic and relapse blasts in early but not late relapses [28]. This suggests that early relapses occur as the result of a sub-clone already present at original diagnosis. Intriguingly, GEP analyses suggest that this clone is highly proliferative and thus the mechanisms by which it resists chemotherapy and allo-SCT remain to be elucidated. In contrast, in late relapses, there are at least two possibilities. There is evidence to suggest that these relapses are derived from the same ancestral clone that gave rise to the original leukemia [29, 30]. In essence, this is a second leukemia but as result of its origin, it retains the chemosensitivity of the original disease. Thus, these patients respond well to chemotherapy. Within this group of later relapses, we know that there are patients who show a slower clearance of disease. These patients often require allo-SCT to sustain remission. As discussed later, these differences may be accounted for by germline polymorphisms in genes regulating drug metabolism.

### 2.4 Why Do Relapses Occur at Extramedullary Sites?

A conundrum in relapsed ALL is the high incidence of recurrence at extramedullary sites such as the central nervous system (CNS). Such relapses tend to occur late and can be either isolated or combined with a bone marrow relapse. Curiously, the outcome of combined or isolated extramedullary relapses is better than isolated marrow relapse. This is puzzling as in most cases of isolated extramedullary disease it is possible to detect low levels of marrow involvement using molecular techniques [31]. Why and how do lymphoblasts enter extramedullary compartments? The clue

lies perhaps in the observation of a striking dichotomy in CNS disease incidence between diagnosis and relapse. While CNS disease is a rare feature of *de novo* ALL, it is seen in around 30% of ALL relapses [19]. It could thus be argued that this phenomenon is selected for by chemotherapy [32]. One possibility is that residual leukemic cells, protected by interactions with the marrow microenvironment, proceed to breach endothelial-matrix barriers and infiltrate extramedullary niches. It is likely that within these sanctuary niches, cells are protected from chemotherapy-induced cell death and give rise to extramedullary recurrences.

## 2.5 How Do We Account for the Heterogeneity in Treatment Response?

Heterogeneity in response to any single drug is commonly observed in patients with ALL. There is evidence to suggest that this is considerably influenced by host genome polymorphisms that regulate drug handling. Polymorphisms relating to increased drug clearance may be responsible for a slower clearance of disease [33–35]. Leukemic blasts may also contribute to the variations in therapeutic response. An example is the enzyme thiopurine *S*-methyltransferase (TPMT). The metabolism of thiopurines is regulated by TPMT. Lower levels of the enzyme are associated with higher toxicity and better outcomes. *TPMT* is located on chromosome 6p. Duplication of this region in the somatic genome can result in high levels of the enzyme in lymphoblasts, which are then able to clear the drug more rapidly [36]. Thus, the tolerated dose of thiopurine may be insufficient to kill blast cells in such cases. More recently, lymphoblasts have been shown to produce proteases capable of inactivating asparaginase [37].

## 2.6 What Is the Role of the Tumour Microenvironment?

Host-tumour interactions are clearly an important component of the spectrum of mechanisms leading to therapeutic failure. Mesenchymal stem cells and haematopoietic stem cell (HSC) niches may provide a protective marrow microenvironment for leukemic cells [38]. As remarked earlier, intrinsically chemosensitive ALL blasts that have the ability to migrate to HSC niches may weather the chemotherapy storm under the umbrella of the microenvironment and re-emerge to cause disease recurrence. These patients may respond to allo-SCT, where ablative conditioning creates an empty marrow niche, thus removing the protective microenvironment. The niche is then colonised by donor HSCs that are presumably able to outcompete residual leukemic cells. Some leukemic cells are capable of modifying the HSC niche to their own advantage, creating their own microenvironment and displacing normal haematopoietic progenitors [39]. Clearly such disease is likely to be incurable with conventional chemotherapy and

allo-SCT. As illustrated by the success of tyrosine kinase inhibitors in Ph+ ALL, such leukemias require targeted therapy. In this context, it is entirely plausible that the same signalling mechanisms that facilitate long-term survival of blasts also facilitate disease progression and extramedullary spread. If so, targeting these survival pathways may prevent disease recurrence.

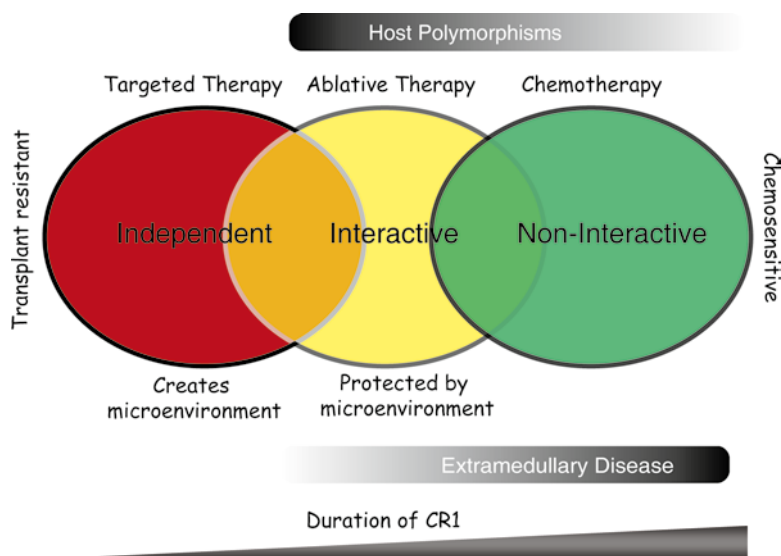
## 2.7 How Do We Discover Novel Biological Targets?

Transcriptional profiling has been shown to be predictive of *in vitro* chemosensitivity [40], as well as the rapidity of response to therapy [41, 42]. However overexpressed genes often lie at the end of a regulatory cascade and it is difficult to ascertain which if any of these genes are directly responsible for therapeutic failure. Additionally, global profiling, even if multi-omic and integrated, does not intrinsically have the resolution to detect expression signatures of minor sub-clones that later account for relapse. Yet, despite these limitations, microarray platforms are already aiding discovery of potential adverse prognostic markers amenable to therapeutic targeting. Aberrant kinase activity has recently been identified as a recurring feature of high-risk disease. Detailed analyses by a number of groups [43–46] show that as a result of either a somatic translocation or deletion, some patients overexpress the cytokine receptor, cytokine receptor-like factor 2 (CRLF2). CRLF2 overexpressing patients have a significantly worse outcome [43]. GEP analyses shows that these patients have an expression signature similar to that seen in Ph+ ALL [47, 48], which includes the adverse-risk Ikaros deletion [43]. More importantly CRLF2 overexpression is associated with somatic activating Janus family kinase (JAK) mutations [49]. These studies suggest that Ikaros, JAK and CRLF2 aberrations cooperate in leukemogenesis and targeting the JAK-STAT signalling mechanism may be an attractive therapeutic strategy.

## 2.8 ALL: A Conceptual Model for Treatment

Figure 2.1 is a proposed conceptual model that integrates lymphoblast characteristics and reciprocal host-tumour interactions to establish a biological and therapeutic paradigm in childhood ALL. Using this model, disease may be categorised into three broad groups:

- Group 1. Highly proliferative, stroma-independent and exquisitely chemosensitive blasts.
- Group 2. Intrinsically chemosensitive blasts that evade chemotherapy by interacting with the host microenvironment; ablative allo-SCT here removes stromal chemoprotection and is curative. Inhibitors capable of disrupting adhesive tumour-stroma interactions (e.g., CXCR4 antagonists) may also have a role here [50].



**Fig. 2.1** An integrated disease paradigm for therapy in childhood ALL. See text for details

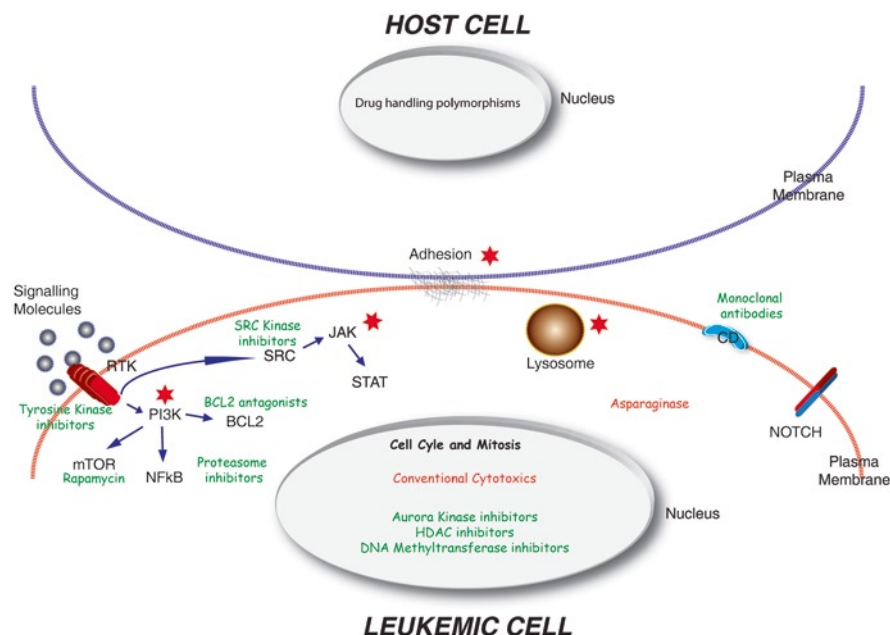
Group 3. Blasts that evade chemotherapy by establishing stroma-independent tumour niches; ablative allo-SCT is ineffective in this group and strategies that target key survival mechanisms are required, exemplified by the ABL tyrosine kinase inhibitors in Ph+ ALL.

Powerfully intersecting with this model are host germline polymorphisms that determine drug disposal and tolerance.

## 2.9 New Molecular and Cellular Treatment Targets

As remarked previously, with the notable exception of asparaginase, all cytotoxic agents including steroids essentially target nuclear mechanisms. The cell nucleus continues to be a focus of drug targeting and many compounds in this class have entered early clinical trials. This includes a number of new nucleoside analogues, the aurora kinase inhibitors that target mitotic spindles [51] and the inhibitors of histone deacetylases [52] and DNA methyltransferases [53, 54] that target the dysregulated transcriptional programme in ALL blasts.

The chapters in this monograph discuss a number of alternative approaches targeting cellular processes and molecules identified in Fig. 2.2. The alternative approaches may be loosely categorised as below. Not all targets have well identified pre-clinical agents in phase I trials.



**Fig. 2.2** A schematic representation of molecular and cellular targets of therapy in acute lymphoblastic leukemia. The lymphoblast nucleus is the principal therapeutic target. Tyrosine kinase inhibitors suppress constitutively activated receptor (e.g., FLT3) or downstream cytoplasmic tyrosine kinases (JAK, SRC, ABL1). Leukemic cell apoptosis is enhanced by suppressing mTOR kinase activity (Rapamycin and analogues) or by NFκB-mediated proteasome inhibition. Non-classical (lysosomal) death pathways may also be triggered by BCL2 family antagonists or by antibody ligation of surface molecules. Monoclonal antibodies typically mediate leukemic cell clearance by activating immune effector mechanisms or by disrupting stromal adhesion. Targeting activated Notch signalling is a potential strategy in T-lineage disease. Asparaginase is unique in its cytotoxicity, selectively perturbing blast cell protein synthesis through substrate depletion. Response to therapy is strongly influenced by host germline polymorphisms governing drug disposal and tolerance. *ABL* Abelson murine leukemia viral oncogene homolog tyrosine kinase 1; *BCL2* B-cell lymphoma 2 family of antiapoptotic molecules; *CD* cluster of differentiation antigens; *HDAC* histone deacetylase; *JAK* Janus family tyrosine kinases; *FLT3* FMS-like tyrosine kinase receptor 3; *mTOR* mammalian target of rapamycin; *NFκB* nuclear factor kappa-light-chain enhancer of activated B cells complex of proteins; *PI3K* phosphoinositide-3-kinase; *RTK* receptor tyrosine kinase; *SRC* sarcoma protooncogene family of tyrosine kinases; *STAT* signal transducer and activator of transcription family of proteins. Stars indicate targeting agents in clinical trials in other diseases but not yet in ALL. Additional details in text

### 2.9.1 Steroid-Sensitising Adjuvants

Steroid resistance may be overcome by antagonists of the mammalian target of rapamycin (mTOR) kinase or by pro-apoptotic small molecules. mTOR inhibitors have shown promise as steroid-sensitising agents, operating through down-regulation of the antiapoptotic BCL2 family molecule, MCL1 [55]. The BCL2 antagonist

Obatoclax too is able to restore steroid sensitivity but appears to do this by activating autophagic necroptosis and thus bypassing a block in mitochondrial apoptosis [56].

### ***2.9.2 Monoclonal Antibodies to Surface Molecules***

Surface molecules on the lymphoblast plasma membrane may be targeted using a number of naked and conjugated antibodies. These antibodies typically mediate blast clearance by binding to cognate proteins and activating cellular or non-cellular immune effectors. Alternatively, these antibodies disrupt the function of target molecules (as in the case of antibodies to integrins) or trigger alternative cell death mechanisms (see below).

### ***2.9.3 Kinase Inhibitors***

As highlighted earlier, dysregulated kinase activity is consistently noted in high-risk ALL. We do not fully understand the mechanisms and molecules responsible for kinase survival signalling in pre-B lymphoblasts. This activation may be constitutive as in the case of activating JAK mutations. Alternatively, aberrant activation may be triggered by homotypic or heterotypic adhesion and maintained by paracrine or autocrine mechanisms [57]. Inhibitors targeting key activated kinases, including the receptor tyrosine kinase, FLT3 and the cytoplasmic kinases, SRC [58] and LYN [59], are now in clinical trials. Similarly, JAK and SYK inhibitors have entered trials for autoimmune and inflammatory diseases but have not yet been tested in childhood ALL. Phosphoinositide-3-kinase (PI3 kinase) inhibitors are gradually entering clinical trials but as this is a large family of kinases, further work is required to clarify the pertinent isoforms in childhood ALL.

### ***2.9.4 Alternative Cell Death Pathway Triggers***

Exploring non-apoptotic cell-death mechanisms as a therapeutic strategy is in its nascence but holds promise. For instance in mature B-cell neoplasms, type II antibodies directed against surface CD20 molecules trigger cell death by destabilising lysosomes, leading to intracellular lysosomal leak and caspase-independent cell death [60]. Our observations suggest that aberrant lysosome trafficking is a feature of ALL blast cells and targeting lysosomal hydrolases is an approach that merits investigation. Proteasome inhibitors may similarly operate through both apoptotic and non-apoptotic cell death triggers and are discussed in detail in a later chapter.



### 2.9.5 Others

A number of alternative approaches also have the potential to be successful. Relapses in precursor T-lineage ALL (T-ALL) are an especial therapeutic challenge. More than 50% of T-ALL harbour activating NOTCH1 mutations. The enzyme  $\gamma$ -secretase catalyses the activating cleavage of the NOTCH1 receptor and  $\gamma$ -secretase inhibitors have entered clinical trials.  $\gamma$ -Secretase is also required for the maturation of the intestinal mucosa and thus gut toxicity is dose limiting, though there is evidence from a murine model that this may be overcome by the concomitant use of steroids [61].

## 2.10 Concluding Remarks

After decades marked by a dearth of new agents, the recently invigorated drug pipeline is an exciting development in the treatment of childhood ALL. But this presents its own challenges. How do we integrate these new agents within contemporary treatment protocols? How do we optimally investigate these drugs in clinical trials? How do we examine their specific effects in the context of multi-agent chemotherapy? And importantly, how do we make these agents available to resource-constrained populations? There are no easy answers as yet and radical unorthodox approaches are probably necessary.

The issue of germline polymorphisms has not been addressed in this chapter or discussed elsewhere in the monograph. These polymorphisms are likely key determinants in eventual treatment response and drug toxicity. Suffice to say, we just do not know enough about the different pathways responsible for the degradation of drugs used in childhood ALL as we have not had the tools to investigate this in detail. With the advent of cheaper germline whole genome sequencing, this is set to change. So, to end from where we started, these are heady times for those of us who look after children with ALL. Among the first to show that a cancer can be cured, we as a community can now proceed to demonstrate how an understanding of the biology of the disease can be harnessed to individualise therapy. This will not only lead to more cures and less toxicity but hopefully cheaper and simpler treatment options that can be applied globally.

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