Targeted Therapies for Childhood ALL

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web version: www.all-kids.org/targetedALL.html (includes updates and links to clinical trials)

Backgound

Current therapies for childhood ALL generally claim an 85% overall survival rate. This is significantly better than the 10% survival rates in the 1960s or the "no survival" rate in the first part of the twentieth century. But the current therapies come with a price, as they are (almost) all "anti-neoplastic", meaning that they kill all dividing cells in the body. Since cancer cells divide more often than most other cells, they are more likely to be killed. But other dividing cells are killed too, leaving the children immunosuppressed, fatigued, plagued with mouth sores and gastrointestinal problems, hairless. The chemotherapies are toxic in other ways too, potentially leading to short and long term effects including cardiac issues, neuropathies, bone loss, avascular necrosis, learning issues, secondary cancers, and more. Some children have radiation in their treatment, which leads to another set of possible short and long term issues.

My son was diagnosed in 1997. I read his treatment plan and cringed: How could we pour all these toxic chemicals into his body for over 3 years? It was surreal watching the nurse administer the medication, then carefully disposing the syringes into toxic waste containers. A PhD student in a research lab working with an analog of one of his medications accidentally got some of it on herself and she thought she was in immediate medical compromise. But we were putting this drug in our son's veins!

It was my belief at that time—1997—that within a decade, the treatments for childhood ALL would advance to much less toxic therapies. Not so. Thirteen years later very little has changed in the treatment plans for ALL. In the standard of care treatment plans, the same toxic drugs are used as in the 1990s. Two additional toxic drugs, nelarabine and clofarabine, have been added to some trials. The only targeted drug is imatinib, which is only used for treatment in cases of the rare sub type, Ph+ childhood ALL. Because of MRD monitoring and the recognition of different sub types of the disease, treatment plans are now somewhat customized to each child's response to treatment and/or disease type. But the bottom line is, my son would have pretty much the same treatment today that he had in 1997, abeit probably it would be even more intense with toxic drugs. My prediction of treatment advances was wrong—and I am not happy about it.

That's why I perked up when I came across an article in the British Journal of Haematology. The title of the article is "Targeting paediatric acute lymphoblastic leukaemia: novel therapies currently in development".[1] This is a review article that covers targeted therapies for ALL that are either still in a research lab or actually out being studied in phase I or II clinical trials.

I was so interested in the targeted therapies article that I decided to write a "lay summary" of it, so that I could better understand the new strategies and keep track of the many new drugs (and their multiple names). I admit, this summary is written primarily for myself, but I am happy to share.

Lay summary of the journal article on targeted therapies

When I hear of a new drug for ALL, I want to know everything about it: Is it another toxic drug? Is it a targeted therapy? What sub types of ALL is it likely to work best on? Is it or was it in a clinical trial? What are the side effects? Sometimes simply recognizing that a drug is new is confounded by the fact that they usually have several names. For instance, imatinib is also known as Gleevec, Glivec, STI 571, or imatinib mesilate. The targeted ALL therapies article lists scores of new drugs: How to keep them straight?

I created the table below by shortening the detailed information in Table I of the review article,[1] including only the drugs currently in trials for pediatric ALL. The majority of these targeted drugs trials are only for relapsed or refractory childhood ALL. And, the treatment plans that incorporate these targeted therapies are generally used in conjunction with one or more of the traditional cytotoxic chemotherapies.

mode of action	*name of drugs*	*use in childhood ALL*
BCR-ABL1 tyrosine kinase inhibition	Imatinib (STI571); Dasatinib (BMS-354825); Nilotinib (AMN107)	in trials (Ph+ ALL)
FLT3 receptor tyrosine kinase inhibition	Lestaurtanib (CEP-701); Midostaurin (PKC-412)	in trials (MLL-rearranged ALL, some T-cell ALLs, and high hyperdiploid ALL)
mTOR kinase inhibitors	Rapamycin (Sirolimus); Temsirolimus (CCI-779)	currently in phase I relapsed ALL trials
aurora kinase inhibitors	MLN8237	phase I/II relapsed ALL trials (currently closed)
multi-kinase inhibitors	Sorafenib (BAY439006)	phase I/II relapsed ALL trials
proteasome inhibitors	Bortezomib (PS-341)	phase I/II relapsed ALL trials
Farnesyltransferase inhibitors	Tipifarnib	was in trials, is not currently in trials
BCL2 antagonists	Obatoclax (GX 15-070)	phase I relapsed ALL trial
Histone deacetylase inhibitors	vorinostat (SAHA)	phase II relapsed ALL
DNA methyltransferase inhibitors	Decitabine	phase I/II relapsed ALL trials
CD Marker Antibodies	Rituximab; Epratuzimab; Alemtuzumab	phase I/II relapsed ALL trials
Conjugated CD Marker Antibodies	CAT8015 (HA22) DT2219ARL BU-12	phase I relapsed ALL trial

Note on clinical trials: to find a clinical trial for a particular drug for childhood ALL, first go to the NCI clinical trials search page.[2] Choose acute lymphoblastic leukemia, child; choose trial/ treatment type "treatment"; then click on "choose from list" next to the drug button. After you have entered the drug of choice, go to the bottom of the page and click on the red search button.

Kinases

Phosphorus has many roles in cells. For instance, it is part of the backbone of DNA, it supplies energy, and it activates proteins that serve as enzymes to catalyze cellular reactions. Activation of an enzyme is a way to control what happens in a cell, in the case we are interested in (cancer), it controls whether or not a cell dies.

Cell processes are usually pathways: one molecule affects another, which affects another, and another, and so on until the final outcome. Many factors feed into each pathway in a complex, convoluted maze of on/off switches (at least, it seems so to the layman!). The article has a great graphic of many of the pathways to cell death that have been studied with the "targeted" molecules noted; if you are interested, I suggest you get a copy of the full article, since I cannot reproduce it here for copyright reasons.

A **kinase** is an enzyme (in this case a protein) that facilitates the transfer of a phosphate group, usually from ATP (adenosine triphosphate) to a substrate. This transfer changes the form of the substrate and signals a cellular event to happen or not to happen. (Note: this is a very brief description: it's a lot more complicated than the previous two sentences!) There are over 500 kinases in humans, serving often as signals in various cell pathways. The ones applicable to ALL targeted therapies are described briefly below.

BCR-ABL1 tyrosine kinase inhibition

Philadelphia positive, or Ph+, ALL is characterized by the transposition of a specific part of chromosome 9 (q34, ABL1) and part of chromosome 22 (q11, BCR). This leads to a "fusion gene" that causes production of an incorrect form of tyrosine kinase protein called BCR-ABL1 TK, a form that cannot be regulated via normal cell pathways. In this case, the unregulated kinase eventually causes a stop in the normal process of cell death through apoptosis. No cell death, and it's a cancer cell.

The BCR-ABL1 TK needs to bind to ATP to work, and clever scientists designed a small molecule now called **imatinib** to compete for the ATP binding site that the mutant kinase requires to be active. Thus, the imatinib prevents the BCR-ABL1 TK from being active and the cell dies, as it is supposed to. Imatinib has an effect on cells that have BCR-ABL1 TK, but it has very little effect on the rest of the cells in the body, because they do not have the mutated tyrosine kinase, BCR-ABL 1, and thus imatinib is a *targeted therapy*.

Some patients' cancers become resistant to imatinib. Second generation tyrosine kinase inhibitors have been developed to overcome this problem; dasatinib and nilotinib are examples of newer tyrosine kinase inhibitors currently in clinical trials. Preclinical in vitro studies showed that nilotinib (AMN107) is more potent than imatinib against CML cells by a factor of 20 to 50.[3]

BCR-ABL1 tyrosine kinase inhibitors are currently used in conjunction with chemotherapy in clinical trials for Ph+ ALL. Only a very small percentage of childhood ALLs are Ph+. Still, this is a very important and successful targeted therapy, if only for a specific sub set.

FLT3 receptor tyrosine kinase inhibition

Another tyrosine kinase implicated in ALL is "FLT3 receptor tyrosine kinase" (FL stands for Fms-like). In some types of ALL, the FLT3 receptor tyrosine kinase is highly expressed and the cell grows out of control. One inhibitor of FLT-3 TK is the small molecule **lestaurtinib** (COP-701), which in culture has been shown to cause death of ALL cells that express high levels of FLT3. Another FLT3TK inhibitor in the pipeline is **midostaurin** (PKS-412).

MLL-rearranged infant ALL is one sub type of ALL that often highly expresses FLT3. Some T-cell ALLs and high hyperdiploid ALL also highly express FLT3.[4]

mTOR kinase inhibitors

Some pediatric ALLs have an "upregulated" (meaning, turned on) pathway to increased cell survival called the the PI3K/AKT pathway. One control point in this pathway is called the "mammalian target of rapamycin", or mTOR for short. mTOR is a serine/threonine protein kinase. **Rapamycin** inhibits mTOR and causes a halt in cell growth. **Temsirolimus** is a second generation mTOR inhibitor; both rapamycin and temsirolimus are in early clinical trials for pediatric ALL. **Everolimus** and **ridaforolimus** are later generation mTOR inhibitors that might be tried against ALL. In patient samples of pre-B cells, everolimus (RAD001) synergized with chemotherapy, radiation, and proteasome inhibitors to kill the cells.[5]

Aurora kinase inhibitors

Some leukemia cell lines (meaning, leukemia cells maintained in culture) show increased expression of Aurora serine/threonine kinases, kinases that regulate cell proliferation through control of mitosis, a step in cell division. **MLN8237** is a small molecule that inhibits Aurora A kinase. It is currently in clinical trials for relapsed/refractory childhood leukemias. In 2010, it showed promise in adult cancers. In the Pediatric Preclinical Testing Program, it shows promise against ALL.[6]

Aurora kinase inhibitiors have been tested both in culture and in humans, and inhibit not only Aurora kinases, but also ABL 1 TK and FLT3 kinases.

Multi-kinase inhibitors

As mentioned above in the Aurora kinase section, some inhibitors target more than one kinase. **Sorafenib** is a multi-kinase inhibitor that works in several different points in a pre-survival pathway. There is some promise that this drug will overcome the acquired resistance that has been observed for selective tyrosine kinase inhibitors (like imatinib). Sorafenib has also been found to increase levels of a tumor necrosis factor. Sorafenib is currently in phase I trials for relapsed/refractory pediatric ALL.

Other targets

Kinases and even enzymes are not the only targets being studied.

Proteasome inhibitors

Proteasomes are large protein complexes that help regulate cell growth/death by selectively degrading certain other proteins. When they destroy a protein called I-kappa-B, the transcription factor NFKB1 goes to the cell nucleus and anti-apototic proteins are activated. In pediatric ALL, NFKB1 is "constitutively" active, and this leads to a stoppage of apoptosis/cell death (no cell death = cancer). **Bortezomib** is a specific proteasome inhibitor that has shown promise in clinical trials of adult leukemias and is now in clinical trials of pediatric leukemias.[7]

Farnesyltransferase inhibitors

The protein RAS is activated in several childhood ALLs; this leads to pro-survival of the cell. RAS is first produced in the cell without a group called "farnesyl isoprene", and it requires the enzyme farnesyltransferase to be activated. Farnesyltransferase inhibitors thus provide a targeted therapy for childhood ALL. **Tipifarnib** was found to moderately decrease farnesyltransferase in phase I clinical trials.[8,9] There is some data that suggest that T-cell leukemias are more sensitive to it than B-cell leukemias. It is not currently in pediatric ALL trials.

Targeting apoptotic pathways

BCL2 antagonists

Programmed cell death occurs through apoptosis, a word that derives from the Greek "dropping off" or "falling off" as in leaves from a tree. One group of regulatory proteins that helps to control apoptosis is the Bcl-2 family. The pan-Bcl-2 small molecule inhibitor **Obatoclax** is currently in phase I trials on childhood ALL.

ABT-737 is another small molecule that inhibits the Bcl-2 family of proteins and it has shown promise in pediatric ALL cell lines with the MLL rearrangement.[10] (In the referenced article, the authors state that significant Bcl-2 expression was detected in all infant leukemia cells investigated.) When used in combination with common drugs administered in ALL therapy, ABT-737 has the ability to enhance the combined toxicity of these drugs against the leukaemia cells in vitro and in vivo.[11]

Epigenetic targets

Histone deacetylase inhibitors

Histone deacetylases (HDAC) are enzymes that remove acetyl groups from histones in nucleosomes and, through a complex pathway, stop the transcription of control proteins (e.g., tumor suppressor genes). With no control, the cell is a cancer cell. HDACs are inhibited by histone deacetylase *inhibitors* (HDACi). In leukemia cells (but not normal cells), HDACi's also *increase* transcription of apoptotic proteins (a good thing). The HDACi **vorinostat** is currently in clinical trials in combination with decitabine (next category).

DNA methyltransferase inhibitors

In ALL, it has been found that certain regions of the DNA are overly covered with methyl groups. The regions of interest are areas that control cell growth, and the "hypermethylation" leads to the cell growing out of control. DNA methyltransferase inhibitors prevent this hypermethylation. 5-Azacytidine (**azacitadine**) is a DNA methyltransferase inhibitor that has been assessed in adult malignancies; the more potent 5-aza-2'-deoxycytidine (**decitabine**) is currently in clinical trials fro childhood ALL. In one case, a pediatric patient with multiply relapsed ALL achieved remission with decitabine.

According to two new papers (this is not in the review article), aberrant DNA methylation occurs in the majority of infant ALL cases with the MLL rearrangement, so maybe DNA methylase inhibitors will prove useful for MLL infant ALL.[12,13]

Non-oncogenic surface targets

CD Marker Antibodies

Parents of children with ALL may be familiar with the term "CD surface markers", which are proteins on the cell surface. For instance, pre-B ALL sub types express CD19 and CD10, while T-cell ALL sub types express CD2, CD7, CD5, or CD3. (CD stands for "cluster of diferentiation".) Researchers have found that monoclonal antibodies (mAb) that target specific B-cell CD markers kill the cells that have the markers, although they are not sure as to the exact mechanism. CD22 is expressed in over 95% of pre-B ALLs; **epratuzumab** is an anti-CD22 mAb; [14] **Rituximab** is an anti-CD20 mAb. Other mABs in the pipeline include **alemtuzumab** and **CMC-544**.

Conjugated CD Marker Antibodies

Monoclonal antibodies (mAb) alone do not always cause cell death. However, a mAb can be connected to a cell-killing (cytotoxic) agent, such as an antibiotic, a bacterial exotoxin, or a radioisotope to form a "conjugated mAb". The conjugated mAb can then snuggle up right next to the targeted cell and deliver the cytotoxic agent.

The following are conjugated CD marker antibodies in the pipeline.

- anti-CD 22: CD 22 mAb conjugated to the antibiotic calicheamicin, named CMC-544 (inotuzumab ozogamicin)
- anti-CD 19: CD 19 mAb conjugated to the antibiotic calicheamicin
- anti-CD22 mAb linked to the pseudomonas exotoxin PE38, named CAT 3888 (BL22)
- anti-CD22 mAb linked to the Pseudomonas exotoxin PE38, named CAT 8015 (HA22) (second generation)
- anti-CD22 immunotoxin HA22 (CAT-8015)
- dual anti-CD22 and anti-CD19 linked to the immunotoxin deglycosylated ricin-A (RFB4-dgA and HD37-dgA), named Combotox
- DT2219 ARL (anti-CD19/CD22 bispecific ligand-directed toxin)
- BU-12 (yttrium Y 90 anti-CD19 monoclonal antibody BU12)

Other target therapy strategies that might soon have drugs in the pipeline

Finally, I am listing some therapies that were discussed in the article but do not yet have drugs in clinical trials. TAM tyrosine kinase inhibitors are studied still in cell lines (in vitro), hopefully these will be useful for childhood leukemias. Gamma secretase inhibitors show promise for T-cell ALL (NOTCH mutations). Securin, a protein involved in cell division, is a target for inhibition. Others: heat shock protein inhibitors (tanespimycin, alvespinycin), TRAIL receptor agonists (lexatumumab, mapatumumab, survivin inhibitors, JAK tyrosine kinase inhibition (Down syndrome and Latino/Hispanic patients), Survivin inhibitors, BiTE antibodies, Chimeric T-cell receptors.

Conclusions

As presented in the article, there are quite a few promising targeted therapies in the pipeline for use in treating childhood ALL. So far, no single one has stood out as the cureall for all types of ALL, but it is encouraging that progress is being made, and perhaps one of these up-and-coming treatments will make a big difference in all subtypes of ALL.

As promising as they are, the targeted therapies present challenges. For instance, imatinib has been in treatment plans for a long time, and patients do build up a resistance to this drug. For instance, other pathways in the cell can upregulate to compensate for the inhibited protein. A problem with cell surface protein targeting is the likelihood of altered cell surface receptor expression after the therapy ends, perhaps leading to the selection of a population of cells with high resistance.

Another issue is that the therapies are not absolutely targeted therapies, meaning that they do affect some normal cells in the body. Thus, side effects both on treatment and years after treatment might still be an issue. Such late effects are not anticipated, but only time will tell.

In the conclusion of the article is the statement "Despite the possible secondary effects of targeted therapies, these novel treatments have great potential." They continue on to state that childhood ALL is a "heterogeneous group of cancers . . . [allowing] development of novel treatments based on the exact specificaitons of the disease, such as treating pre-B cell leukemia with an anti-CD22 mAb, treating MLL-rearranged leukemia with a FLT3 inhibitor, or treating T-cell ALL with a gamma-secretase inhibitor." Thus, someday a child's treatment will be specifically targeted, according to the subtype of the cancer.

My thoughts, in conclusion

On a personal level, I am both encouraged and discouraged. Encouraged because a good number of strategies for non-toxic therapies are in the works for childhood ALL. Discouraged because these treatments will not soon rid childhood ALL treatment plans of toxic therapies—currently, in clinical trials, the therapies are still being used in conjunction with conventional chemotherapy regimens. I was hoping for a "magic bullet". I was hoping that soon a newly diagnosed child would swallow a few pills, go home, and be leukemia free.

Hopefully the day will soon come where the treatment for childhood ALL will not be as harsh as it is today. A newly diagnosed child's leukemia cells would be tested immediately, the exact

specification of the disease would be determined, and a non-toxic treatment plan initiated that would give a 100% cure rate with no long term effects.

We can hope. I for one am glad to know that the researchers are indeed working towards this goal.

[1] *Targeting paediatric acute lymphoblastic leukaemia: novel therapies currently in development*. Alisa B. Lee-Sherick, Rachel M. A. Linger, Lia Gore, Amy K. Keating and Douglas K. Graham, British Journal of Haematology, Article first published online: 31 Aug 2010.

[2] http://www.cancer.gov/clinicaltrials/search

[3] *Nilotinib in Imatinib-Resistant CML and Philadelphia Chromosome-Positive ALL*. Hagop Kantarjian et al., N Engl J Med 2006; 354:2542-2551, June 15, 2006.

[4] *Targeting FLT3 in primary MLL-gene–rearranged infant acute lymphoblastic leukemia*. Ronald W. Stam et al., Blood, 1 October 2005, Vol. 106, No. 7, pp. 2484-2490.

[5] *The mTOR inhibitor RAD001 (Everolimus) synergizes with chemotherapeutic agents, ionizing radiation and proteasome inhibitors in pre-B ALL*. Philip O. Saunders et al., Haematologica 2010, 10.3324, Published online 15 October 2010.

[6] *Initial testing of the aurora kinase a inhibitor MLN8237 by the Pediatric Preclinical Testing Program (PPTP)*. John M. Maris et al., Pediatric Blood & Cancer, Volume 55, Issue 1, pages 26–34, 15 July 2010.

[7] http://www.cancer.gov/clinicaltrials/NCT00440726

[8] *Phase 1 trial and pharmacokinetic study of the farnesyl transferase inhibitor tipifarnib in children and adolescents with refractory leukemias: A report from the Children's Oncology Group*. Brigitte C. Widemann et al., Pediatric Blood & Cancer, Volume 56, Issue 2, pages 226–233, February 2011.

[9] *In vitro profiling of the sensitivity of pediatric leukemia cells to Tipifarnib (ZarnestraTM): Identification of T-cell ALL and FAB M5 AML as the most sensitive subsets*. Bianca F Goemans et al., Blood, 15 November 2005, Vol. 106, No. 10, pp. 3532-3537.

[10] *Cytotoxicity, drug combinability, and biological correlates of ABT-737 against acute lymphoblastic leukemia cells with MLL rearrangement*. Jayanthan A et al., Pediatr Blood Cancer. 2011 Mar;56(3):353-60.

[11] *Activity of vincristine, L-ASP, and dexamethasone against acute lymphoblastic leukemia is enhanced by the BH3-mimetic ABT-737 in vitro and in vivo*. Kang MH et al., Blood. 2007 Sep 15;110(6):2057-66.

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[13] *Promoter hypermethylation in MLL-r infant acute lymphoblastic leukemia: biology and therapeutic targeting*. Schafer E et al., Blood. 2010 Jun 10;115(23):4798-809.

[14] *Chemoimmunotherapy reinduction with epratuzumab in children with ALL with marrow relapse: A Children's Oncology Group (COG) pilot study (ADVL04P2*. E. A. Raetz et al., Journal of Clinical Oncology, 2007 ASCO Annual Meeting Proceedings (Post-Meeting Edition). Vol 25, No 18S (June 20 Supplement), 2007: 951.