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## Immunotherapy of Childhood Cancer: From Biologic Understanding to Clinical Application

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### Abstract

**Purpose of review**—Most children with cancer can be cured with combination regimens of chemotherapy, radiation, and/or surgery. However, standard therapies are toxic to normal tissues, cancer cells commonly develop resistance to chemotherapy, and relapsed malignancy is a leading cause of mortality in pediatrics. Elucidation of the principles of the normal immune response and tumor biology, coupled with technological developments, have led to important advances in the field of cancer immunotherapy. This review summarizes the biologic basis of cancer immunotherapy and highlights recent examples of progress in the application of novel humoral and cellular immunotherapies to children and adolescents with malignancy.

**Recent Findings**—Clinical trials of immunotherapy for pediatric cancer have recently been initiated. To date, most immune-based therapies have been well tolerated and some have shown clinically significant activity against specific refractory high-risk malignancies.

**Summary**—Recent clinical trial results provide proof-of-principle that cancer immunotherapy has the capacity to overcome chemotherapy resistance without the usual toxicities associated with cytotoxic regimens. Immunotherapy holds promise in the treatment of children and adolescents with cancer and has the potential to improve both survival and quality of life.

### Keywords

cancer; childhood; immunotherapy; monoclonal antibodies; tumor vaccines

### Introduction

Although most children with cancer are cured, the limits of standard cytotoxic therapies appear to have been reached for many pediatric malignancies.<sup>1</sup> Non-specific side effects of treatment are substantial,<sup>2</sup> and death from relapse remains a leading cause of mortality in pediatrics.<sup>3</sup> Scientific discovery in immunology and tumor biology and related technological advances have facilitated dramatic developments in the field of cancer immunotherapy. This review summarizes biologic principles that underlie immunotherapy and highlights recent examples

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of the application of novel immunotherapeutic approaches to the treatment of cancer in children and adolescents.

### The biology of immune responses

The normal human immune system is composed of two primary components: *innate* and *adaptive* immunity.<sup>4,5</sup> Adaptive immunity is further subdivided into *humoral* and *cellular* arms. Importantly, these elements of the immune system are highly interdependent and interconnected.<sup>6</sup> [Figure 1] Innate immune responses do not require prior exposure to target antigens. Effector cells, including phagocytic and cytotoxic leukocytes and cytokines play important roles in the first line of defense against microorganisms and in the activation of the adaptive immune response. There is evidence to indicate that the innate immune system can be directed against malignant cells.<sup>7</sup> However, this approach to cancer immunotherapy has lagged behind the application of adaptive immune mechanisms. Clinical trials of activators of innate immunity in pediatric cancers have only recently begun and these will not be reviewed here. The adaptive immune system represents a complex network of afferent and efferent signals and effectors responsible for maintaining long-term immunity against infectious pathogens and foreign antigens. The humoral arm is constituted by B-lymphocytes responsible for the production of antibodies, while cellular immunity is mediated primarily by CD4+ and CD8+ T cells. Both components of the adaptive immune system have been successfully exploited in the treatment of cancer, and each will be considered separately.

**Cancer-associated antigen targets for immunotherapy**—A wide array of antigens can serve as targets for immune responses against cancer in experimental systems and in humans. These include specific chromosomal translocation fusion proteins, tissue- or cell-lineage-specific differentiation antigens, gene products that are over-expressed by malignant cells, and histocompatibility antigens.<sup>8,9,10</sup> At the same time, cancer cells can elude immune responses in a variety of ways. Because the kinetics of immune-mediated killing might be inadequate to control rapidly proliferating cancer, reducing tumor burden to a state of minimal residual disease (MRD) prior to the initiation of immunotherapy is often utilized in attempt to overcome this disparity. Cancer cells can also evade immunologic recognition by a number of well-described mechanisms. Malignant cells may have diminished or absent expression of cancer-associated antigens and/or critically required immune co-stimulatory molecules (see below),<sup>11,12</sup> produce immunosuppressive soluble factors or stimulate the production of immune suppressor cells, and express antigens that induce cell death (apoptosis) of immune effectors. Furthermore, cancer-associated antigens are often weakly immunogenic or overexpressed self-antigens, leading to weak immune responses due to selection events in the thymus early in life, and peripheral anergy. To augment anti-cancer immune responses, malignant cells can be modified to increase their immunogenicity, the immune system can be activated towards cancer-associated antigen targets, and tumor-associated suppressor cells can be depleted. All of these strategies are currently undergoing study in cancer immunotherapy trials.

### Humoral Immunity and Antibody-Based Therapeutics of Cancer

B-lymphocytes produce five classes of antibodies, or immunoglobulin (Ig) molecules (IgA, IgD, IgE, IgG, IgM). IgG secreted by memory B cells is the antibody with the highest concentration in circulation. This molecule is composed of two longer (*heavy*) chains and two shorter (*light*) chains. [Figure 2] The specificity of antigen binding is determined by the amino acid sequence of the *variable region* of the IgG molecule. After initial exposure to the cognate antigen, B cells produce IgM, which is followed by class switch and production of IgG of the same specificity.<sup>4</sup>

## Monoclonal antibodies against human differentiation antigens

There has been dramatic progress in the clinical development of MoAb-based cancer therapeutics over the past 2 decades. Köhler and Milstein first demonstrated that monoclonal antibodies (MoAb) directed against specific human differentiation antigens could be generated from hybridomas derived from immunized mice,<sup>13</sup> which made possible the large-scale production of such reagents for therapeutic use in humans. Subsequently, it was reported that fragments of antibody variable domains (Fv) could be linked together to make recombinant proteins capable of antigen binding.<sup>14</sup> Methodologies have since been developed to produce fully human MoAbs and their fragments for clinical use and to generate humanized constructs with reduced immunogenicity. [Figure 2]

## Monoclonal antibody-based therapies for cancer

For effective MoAb-targeting, the cognate antigen should be expressed in relatively high levels on the surface of the malignant cells and there should be limited-to-no expression on normal tissues. Ideally, there should also be minimal shedding of antigen from the cell surface, since high levels of free antigen could serve as a decoy, thus diminishing MoAb binding to the target.

MoAbs have the potential to kill cancer cells through direct and indirect effector pathways. Certain antibody-receptor binding interactions directly inhibit cell growth or induce cell death (apoptosis) through effects on intracellular signaling pathways. Indirect killing can occur by antibody-dependent cellular cytotoxicity (ADCC), complement-activation, and/or cell-mediated cytokine production. The mechanism involved, or whether killing occurs at all, varies with the specific MoAb, antigen and cancer. Importantly, immune-mediated cytotoxicity requires functional immune effector mechanisms, which are commonly deficient in patients with cancer.<sup>15,16</sup>

## Monoclonal antibodies for hematologic malignancies

Hematologic malignancies, the most common pediatric cancers, are excellent candidates for MoAb-based therapeutics.<sup>17</sup> Malignant blasts from patients with leukemia and lymphoma express lineage-specific human differentiation antigens with otherwise limited tissue distribution, and numerous MoAbs have been developed that effectively target such antigens. In 1997, the U.S. Food and Drug Administration (FDA) approved the first MoAb for the treatment of cancer, rituximab (Genentech, Inc. San Francisco, CA, U.S.A.). This agent is a MoAb directed against the B-lymphoid lineage antigen CD20. Rituximab combined with chemotherapy has been demonstrated to improve disease-free survival in adults with non-Hodgkin's lymphoma (NHL).<sup>18</sup> Results of a recent Children's Oncology Group (COG) trial of rituximab with chemotherapy indicate that this agent can be safely administered to pediatric patients with relapsed CD20+ lymphoma and leukemia.<sup>19\*</sup> The COG and the Berlin-Frankfurt-Muenster cooperative group (BFM) are currently conducting clinical trials of rituximab with chemotherapy for children and adolescents with newly diagnosed CD20+ hematologic malignancies. A COG study of a MoAb that targets the B-lineage antigen CD22, epratuzumab (Immunomedics, Inc., Morris Plains, NJ, U.S.A.), in combination with standard chemotherapy for children with acute lymphoblastic leukemia (ALL) is also in progress. Results in the initial cohort treated with an upfront epratuzumab monotherapy phase were recently published.<sup>20\*</sup> The clinical activity of epratuzumab as a single agent was limited and no complete or partial remissions were observed. In this preliminary analysis, the feasibility of giving epratuzumab in combination with standard chemotherapy was demonstrated and the rates of complete remission and clearance of MRD appeared favorable in comparison to historical results with chemotherapy. It is unlikely that MoAbs will have adequate single agent activity to be effective for pediatric hematologic malignancies when used alone. However, rare cases of complete remissions in adults and children with ALL have been reported with MoAbs targeting CD20, CD33, and CD52.<sup>21,22,23</sup>

## Monoclonal antibodies for solid tumors

Recent success has also been achieved using MoAbs to target antigens expressed on pediatric solid tumors. The ganglioside GD2 is a neuroectodermal-restricted antigen expressed by neuroblastoma. A number of MoAbs targeting GD2 have been studied in children with high-risk neuroblastoma.<sup>24,25,26,27,28,29</sup> The anti-GD2 MoAb 3F8 cleared MRD after autologous SCT in children with stage IV neuroblastoma.<sup>30</sup> The anti-GD2 MoAb ch14.18, in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-2 (IL-2) after autologous SCT, enhanced event-free and overall survival in comparison to standard therapy.<sup>31\*\*</sup> Importantly, this regimen had limited activity in patients with bulky neuroblastoma, consistent with a model wherein immune-based therapies are more effective when administered in the setting of MRD.

The insulin-like growth factor (IGF) signaling pathway plays a role in cell survival in certain pediatric sarcomas.<sup>32,33\*</sup> A number of MoAbs against the IGF-1 receptor are undergoing clinical trial in pediatric and adult patients with sarcomas and dramatic responses have been observed suggesting that IGF-1 receptor blockade can interrupt critical cell survival signals.<sup>32</sup>

The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor is expressed on a variety of cancers, and anti-TRAIL receptor MoAbs have shown activity against pediatric sarcomas in pre-clinical models.<sup>34</sup> A phase I trial of an anti-TRAIL MoAb for children with solid tumors is in progress at the National Cancer Institute (NCI).

CTLA4 is a receptor on the surface of T cells that diminishes immune responses. Blockade of CTLA4 signaling through anti-CTLA4 MoAbs inhibits this suppressive signal and augments T cell mediated immune reactivity. This agent has activity against melanoma and other tumors in adults,<sup>35</sup> and a pediatric Phase I trial is being performed at the NCI.

## Conjugated monoclonal antibodies

The cytotoxicity of MoAbs can be dramatically increased by linkage to toxic moieties such as chemotherapeutic agents, bacterial and plant toxins, and radioisotopes. Conjugating highly cytotoxic agents to MoAbs should improve the therapeutic index since the MoAb directs killing to those cells that express the target antigen, thus limiting non-specific damage to normal tissues. Importantly, MoAb-based agents armed with potentially cytotoxic compounds do not require active immune response mechanisms for activity. As a result, they can be effective even in profoundly immunocompromised patients.

## Chemotherapeutic conjugates

MoAbs have been conjugated to a number of active chemotherapeutic agents. The first MoAb conjugate to receive FDA approval in the treatment of cancer was gemtuzumab ozogamicin (Pfizer, New York, NY, U.S.A.). This agent targets the myeloid antigen CD33 and is linked to calicheamicin, a potent antitumor antibiotic. Clinical trials of gemtuzumab ozogamicin have been conducted for children with acute myelogenous leukemia (AML).<sup>36,37\*\*</sup> Approximately 30% of pediatric patients with relapsed AML respond to gemtuzumab ozogamicin as a single agent. Clinical trials designed to assess the efficacy of gemtuzumab ozogamicin in combination with chemotherapy are being conducted by the COG,<sup>38</sup> while the Nordic Society of Paediatric Haematology and Oncology is studying its role as consolidation prior to SCT in children with high-risk AML. This agent has also been used to treat occasional cases of ALL with CD33 expression and anecdotal cases of successful remission induction have been reported.<sup>39</sup>

## Toxin Conjugates

Immunotoxins are engineered proteins consisting of a MoAb-based targeting moiety that mediates cell binding and a toxin that induces cell death upon internalization. [Figure 3] A 38

kD truncated derivative of *Pseudomonas* exotoxin A (PE38) has been used at the NCI to develop recombinant immunotoxins that target human differentiation antigens.<sup>40</sup> A recombinant immunotoxin that targets the B-lineage antigen CD22, CAT-3888 or BL22 (MedImmune LLC, Gaithersburg, MD, U.S.A.), is highly active in adults with B-lymphoid malignancies.<sup>41,42</sup> A recently completed pediatric Phase I study demonstrated an acceptable toxicity profile and clinical activity in children with CD22+ ALL and NHL.<sup>43</sup> A follow-up pediatric trial of a second-generation agent with higher CD22 binding affinity and increased pre-clinical activity,<sup>44</sup> CAT-8015 or HA22, (MedImmune LLC, Gaithersburg, MD, U.S.A.), is in progress.<sup>45\*</sup> Anti-CD22 immunotoxins appear to have synergistic *in vitro* cytotoxicity against childhood ALL blasts when combined with chemotherapy.<sup>46</sup>

### Radioisotope conjugates

MoAbs that target leukemia-associated antigens have been linked to radioactive isotopes, most commonly  $\beta$ -emitters (e.g., <sup>90</sup>Yttrium, <sup>131</sup>Iodine, <sup>186</sup>Rhenium) and less frequently  $\alpha$ -emitters (e.g., <sup>213</sup>Bismuth). These agents are concentrated in the bone marrow and consequently cause severe myelosuppression. Thus, the application of radioimmunotherapy for hematologic malignancies is limited primarily to myeloablative conditioning prior to SCT,<sup>47</sup> and there have been very few trials in the pediatric age group.<sup>48,49</sup> Studies are being conducted with a 3F8-<sup>131</sup>Iodine conjugate for children with GD2-expressing brain tumors.<sup>50</sup>

### Potential side effects and limitations of monoclonal antibody-based therapeutics

In general, most of the MoAb-based therapeutics currently being advanced for clinical use have been well tolerated with markedly reduced risks of organ toxicity in comparison to chemotherapy and radiation. Nonetheless, there are a variety of specific side effects associated with individual MoAbs and immunotoxins. Acute infusion reactions are relatively common, although these can usually be managed or prevented by treatment with antipyretics, antihistamines, and/or corticosteroids. Depletion of normal blood cells that express the target antigen can be expected. For example, rituximab is associated with B cell depletion, humoral immunosuppression, and risk of certain viral infections. Immunotoxins are associated with a number of unique toxicities, for example, vascular leak syndrome.

### Immunogenicity

Despite the creation of human/murine (“humanized”) antibodies, some components of many MoAbs are derived from foreign species. Similarly, toxins represent foreign proteins. Thus, patients may develop antibodies to foreign protein epitopes (i.e., human anti-mouse and/or human anti-toxin antibodies) that bind and diminish or neutralize therapeutic activity. Notably, there may be pre-existing antibodies due to prior vaccination (e.g., *Diphtheria*) or infection (e.g., *Pseudomonas*).

### Cellular Immunity and Cell-Based Therapeutics of Cancer

Cellular immunity is mediated primarily by CD4+ and CD8+ T cells. [Figure 1] T cells recognize antigen as peptides derived from the breakdown of intracellular proteins displayed on the surface of antigen presenting cells (APCs) in the context of major histocompatibility (MHC) antigens.<sup>8,12,51</sup> [Figure 4] The generation of a successful immune response by a naïve T cell requires the interaction of an appropriate T cell receptor and MHC-presented peptide antigen, known as *Signal #1*, along with a co-stimulatory signal, known as *Signal #2*. [Figure 4] This second signal can be via an appropriate cytokine or through by cell-to-cell interaction through one of a variety a co-stimulatory molecule/ligand pairs (e.g., CD40/CD40L, B7 family/CD28). Activation of the T cell receptor in the absence of appropriate co-stimulation results in antigen-specific tolerance.<sup>5</sup>

## Allogeneic stem cell transplantation and the graft-versus-leukemia effect

Allogeneic SCT represents the most commonly employed and well-proven form of cellular immunotherapy for childhood cancer. SCT can be curative for most subtypes of pediatric hematologic malignancies.<sup>52,53</sup> A T cell-mediated allogeneic immunologic reaction, the *graft-versus-leukemia* (GVL) effect, reduces the risk of relapse after SCT.<sup>54,55,56,57,58,59,60</sup> Among the most dramatic demonstrations of the potency of T cell immunotherapy in the treatment of cancer is the effect of donor lymphocyte infusions (DLI) for relapsed leukemia after SCT.<sup>61,62,63,64,65</sup> [Supplemental Figure 1] DLI induces complete remissions in more than 70% of adults and children with chronic phase chronic myelogenous leukemia (CML). This approach, however, is far less effective in other subtypes of leukemia, especially ALL. There are a number of possible mechanisms for the resistance of ALL to DLI. For example, unlike myeloid cells, B-lineage lymphoblasts have very low expression of T cell co-stimulatory molecules (e.g., B7 family). Consequently, ALL blasts present antigens poorly and they may induce T cell anergy.<sup>66</sup> Importantly, a major complication of DLI, and SCT in general, is graft-versus-host disease (GVHD), which represents an allogeneic reaction of donor T cells against normal host tissues. Thus, a key aim of cellular immunotherapy after SCT is to implement strategies that induce GVL in the absence of GVHD.

## Cancer vaccines

Cancer vaccines are designed to generate sustained responses to a specific antigen through the formation of immunologic memory. A variety of approaches have been utilized in attempt to stimulate T cell immune responses toward cancer-associated antigens *in vivo*.

## Peptide vaccines

Peptides can be readily synthesized for clinical use as a source of tumor-associated antigens for cancer vaccines. Immunologic and clinical responses, including complete remissions, have been observed in adults with leukemia and solid tumors treated with peptide vaccines targeting the myeloid antigen proteinase<sup>67,68</sup> and the Wilms tumor-1 (WT1) protein, a transcription factor expressed by a wide array of malignancies.<sup>69,70</sup> Importantly however, applicability of peptide vaccines is restricted by the specific antigenic epitopes and HLA binding motifs. This is particularly limiting in pediatric cancers, which are far less common than malignancies in adults.

## Dendritic cells and artificial antigen presenting cells for cancer vaccines

Dendritic cells are specialized APCs that play a critical role in the adaptive immune response, [Figure 1] and can be generated from peripheral blood monocytes *ex vivo* for use in cancer vaccines.<sup>71</sup> Additionally, artificial APCs can be engineered to express key co-stimulatory molecules for immunotherapy trials.<sup>72</sup> Peptides, nucleic acids, proteins, and tumor lysates can all be used to prime APCs to present cancer-associated antigens, which can then be administered as a vaccine to direct a T cell response towards cancer-associated targets. [Supplemental Figure 2] Clinical responses to APC-based vaccines have been reported in pediatric trials, with one patient with fibrosarcoma achieving a complete response to a tumor lysate-pulsed dendritic cell vaccine.<sup>73,74</sup> Favorable overall survival was reported in a recent study of autologous T cell infusion and peptide-pulsed dendritic cell vaccine targeting the translocation breakpoints in pediatric sarcomas.<sup>75\*</sup> A follow-up trial using autologous tumor lysate as the antigenic source is in progress at the NCI. A novel allogeneic vaccine trial that utilizes WT1 peptide-loaded dendritic cells generated from healthy SCT donors is being conducted at the NCI for children and adults with WT1-expressing hematologic malignancies. Vaccines are administered along with DLI in attempt to augment the GVL effect for individuals with persistent or relapsed disease after allogeneic SCT. [Supplemental Figure 2]

Autologous and allogeneic cancer cells can also be modified to function directly as APCs. As an example, blasts from children with B-precursor ALL can be rendered capable of presenting antigens by incubation with the co-stimulatory molecule CD40 ligand and the cytokine IL-4. Thus, ALL blasts can be transformed into APCs, overcoming their ability to induce T cell energy.<sup>66</sup> Similarly, autologous and allogeneic neuroblastoma cells transduced with T cell activating cytokines have been utilized in vaccine trials in pediatric patients with neuroblastoma and response rates of 10 to 20% have been reported.<sup>76,77,78</sup>

### **Adoptive immunotherapy with ex vivo expanded T cells**

As noted above, the efficacy of T cell-based immunotherapeutic approaches may be limited by the inability to rapidly generate large numbers of antigen-specific T cells *in vivo*. Effective approaches to generate, expand, and activate large quantities of activated target-directed T cells *ex vivo* for use in adoptive immunotherapy have been developed,<sup>79</sup> and durable complete responses have been observed in adults with chemotherapy-resistant metastatic melanoma.<sup>80\*\*</sup> Studies of genetically engineered cytotoxic T cells with specificity against antigens expressed by pediatric solid tumors are underway at the NCI, and dramatic responses in pediatric patients have recently been observed (unpublished results).

### **Potential side effects and limitations of cellular immunotherapies**

To date, vaccine therapies have been well tolerated, with primarily only minimal injection site pain, erythema and/or induration observed. Immunotherapies that induce a strong T cell response may be associated with autoimmunity.<sup>81</sup> Allogeneic lymphocyte infusions carry the risk of GVHD and transfusion-related side effects.

## **Combined Approaches to Cancer Immunotherapy**

Immunotherapy trials commonly incorporate multiple components designed to maximize the immunologic response.

### **Chimeric Antigen Receptors**

The effectors employed in adoptive therapy can be specifically directed using MoAb-based targeting to engage cancer cells via gene therapy techniques. Genetically engineered chimeric antigen receptors (CARs) have been designed to enable immune effectors to bind to and induce direct cytotoxicity against malignant cells that express differentiation antigens.<sup>82,83,84,85</sup> T cells and NK cells engineered with CARs directed to leukemia-targets (e.g., B-lineage antigen CD19)<sup>86</sup> and solid tumor antigens (e.g., GD2)<sup>87</sup> are currently undergoing study at a number of pediatric centers including the NCI, St. Jude Children's Research Hospital, and the Baylor College of Medicine. EBV-specific cytotoxic lymphocytes engineered to target GD2 were shown to be active against refractory neuroblastoma.<sup>88\*\*</sup>

### **Bi-specific monoclonal antibodies**

MoAb constructs with dual specificity for a leukemia-associated antigen and a surface receptor on immune effector cells (e.g., CD3) can serve to direct cell-mediated cytotoxicity in a fashion similar to CARs. A recombinant anti-CD19/anti-CD3ε bi-specific antibody (blinatumomab, Micromet, Inc., Munich, Germany) has recently been shown to be active in adults with hematologic malignancies.<sup>89\*</sup> Importantly, these agents require functional T cells for activity and therefore might be expected to have increased utility after allogeneic SCT.

### **Immunotherapy combined with standard treatment regimens**

As noted above, clinical trials that integrate immunotherapy with standard cytoreductive regimens are undergoing study. The concept of "consolidative immunotherapy" holds

particular promise in pediatric oncology, where the most common malignancies are usually responsive to front line therapies, and patients with high-risk disease can often be reduced to a state of MRD.

## Conclusions

The majority of pediatric patients with cancer are cured with current standard treatment regimens. However, progress appears to have plateaued and the late effects associated with cytotoxic agents are substantial.<sup>1,2</sup> New approaches that can augment standard therapies and reduce non-specific toxicities are highly sought-after. Discoveries in immunology and cancer biology, along with technological advances, have led to the development of novel immune-based therapies. Immunologically-based cancer therapies have shown promise in pre-clinical models and have been translated into clinical trials. Benefits of immunotherapy in pediatric oncology have recently been realized in specific diseases and numerous approaches hold great promise.<sup>90\*\*</sup> Future studies will help define the optimal approaches and combination regimens. In addition, the effectiveness of immune-based therapies for childhood cancer is expected to increase with the implementation of strategies that can hasten immune reconstitution after standard treatment.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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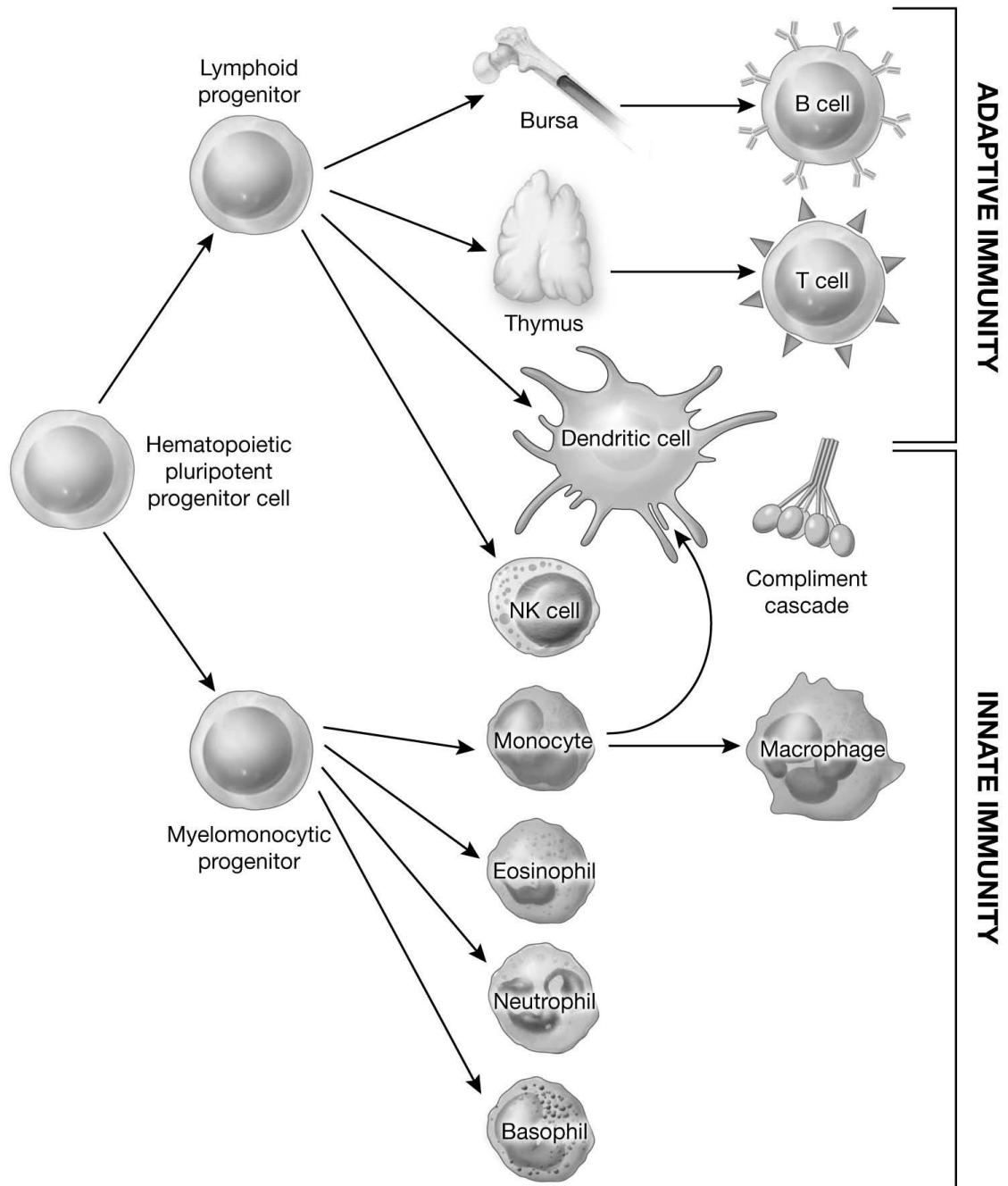
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whether immunotherapy was administered or not, a 5-year overall survival rate of 31% was observed. For those who received immunotherapy, the 5-year overall survival was 43%, which was favorable compared to historical controls.

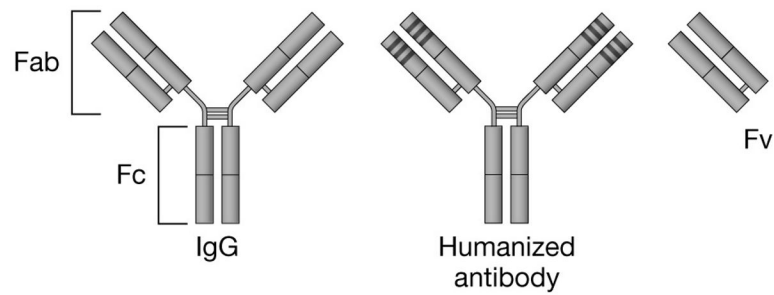
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**Figure 1. Components of the innate and adaptive immune system**

*Adapted from:* Mackall CL, Sondel PM: Tumor immunology and pediatric cancer. In Pizzo, P.A. and Poplack, D.G. (eds) **Principles and Practice of Pediatric Oncology**, 6th edition. Philadelphia, PA, Lippincott Raven Publishers, 2009 (in press).<sup>6</sup>

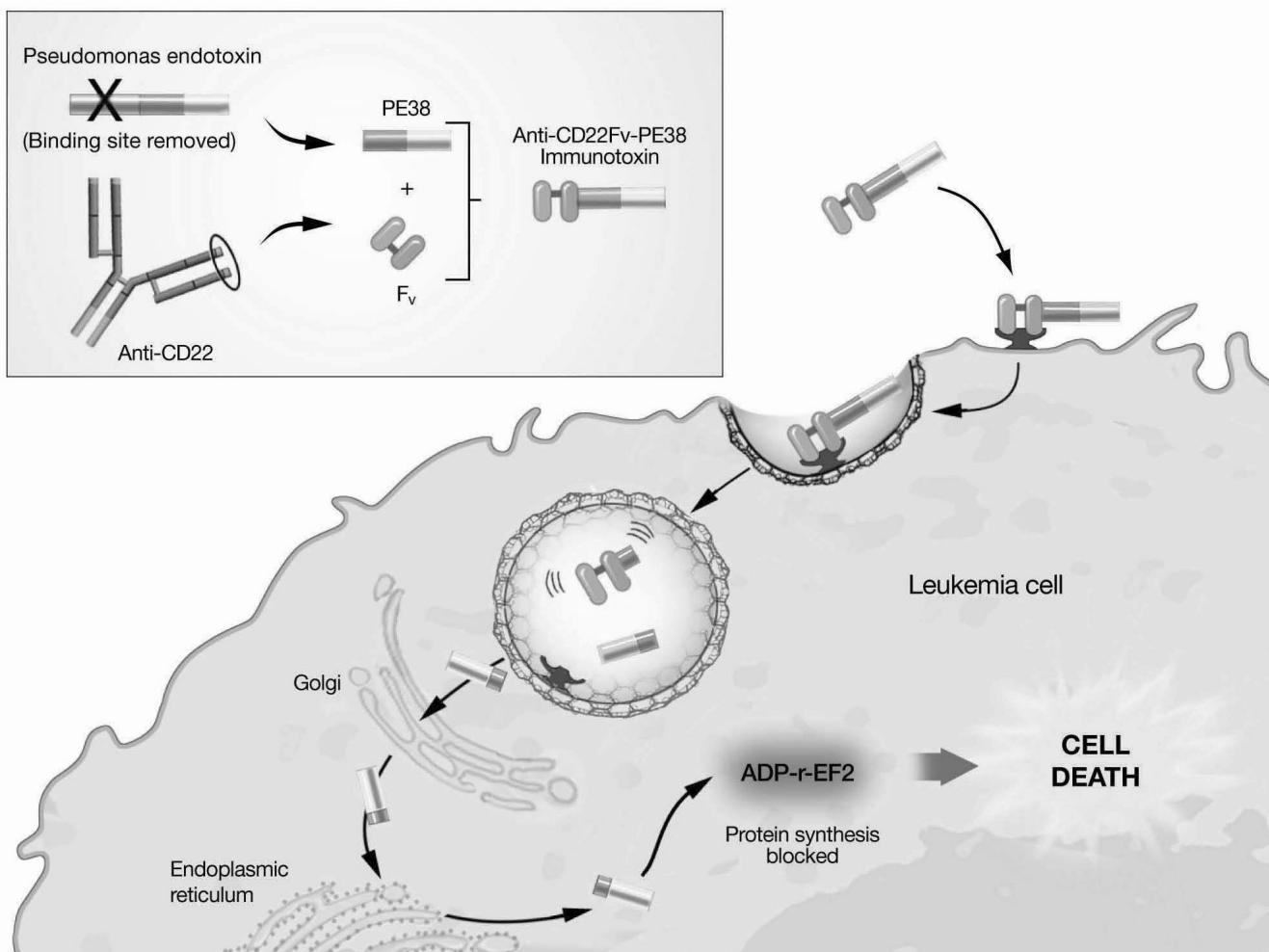


**Figure 2. Structure of immunoglobulin and monoclonal antibody fragments**

The immunoglobulin-G (IgG) molecule is composed of two longer (*heavy*) chains and two shorter (*light*) chains that are connected by disulfide bonds. The amino acid sequence of the variable (*v*) ends of each of the four chains determines the specificity of antigen binding. Humanized monoclonal antibody constructs consist predominantly of human amino acid sequences, with the exception of the three hypervariable complementarity-determining regions (CDR), which retain the foreign-specie sequences critical for antigen binding. The CDR domains are indicated by the dark bands at the end of the antigen-binding portion of the molecule.

*Fab*: Antigen binding fragment; *Fc*: Crystallizable or complement fixing fragment; *Fv*: Variable fragment

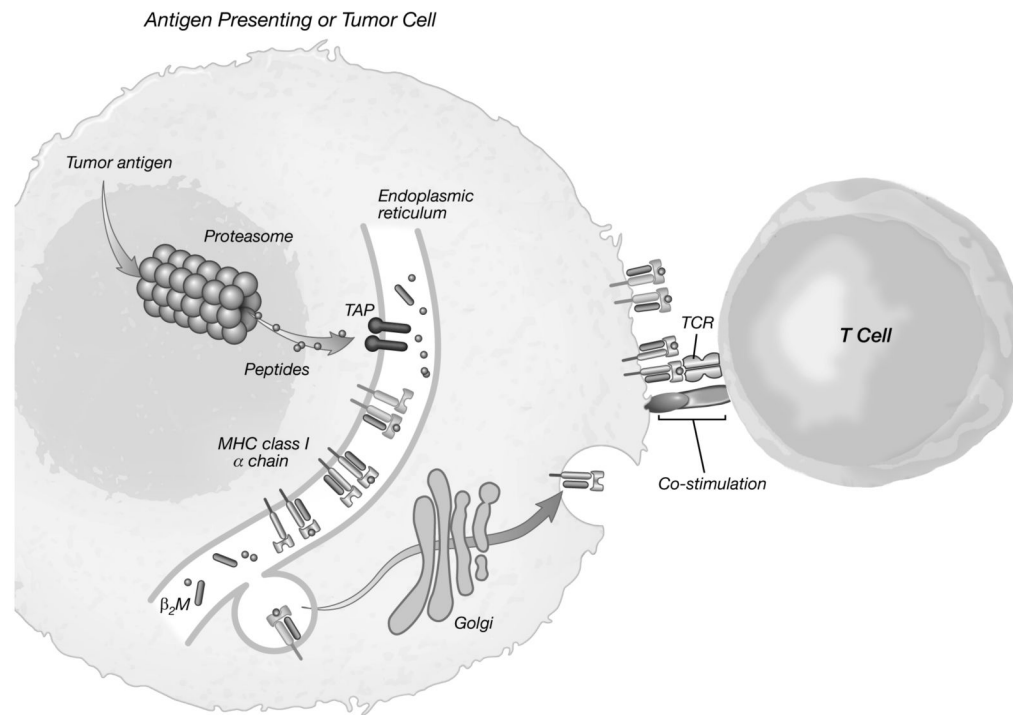




**Figure 3. Pseudomonas-based immunotoxins: structure and mechanism of cytotoxicity** *Inset:* Full-length *Pseudomonas* exotoxin A (PE) protein contains three functional domains that mediate antigen binding, cytosolic translocation, and cytotoxicity. The recombinant anti-CD22 immunotoxin RFB4(dsFv)-PE38 consists of cloned disulfide-stabilized single chain variable fragment (Fv) from the anti-CD22 monoclonal antibody RFB4 and the PE translocation and killing domains (PE38).

Immunotoxin binding to surface CD22 is followed by internalization through endocytosis. Immunotoxin cleavage occurs in the endosome and the C-terminal fragment is transported to the cytosol. ADP ribosylation (ADP-r) inactivates elongation factor-2 (EF2), which inhibits protein synthesis causing cell death.

*Adapted from:* Wayne AS. Application of immunotherapy in pediatric leukemias. *Curr Hematol Malig Reports* 2009;4(3):159-166.<sup>17</sup>



**Figure 4. The T cell immune response**

T cells recognize antigen as small peptides derived from the breakdown of intracellular proteins displayed on the surface of antigen presenting cells in the context of major histocompatibility (MHC) antigens. Tumor antigens are cleaved by the proteasome into fragment peptides. The peptides transported by the transporter associated with antigen processing (TAP) into the endoplasmic reticulum, where they are loaded onto MHC molecules that are assembled and transported to the cell surface.

Activation of naïve T cells requires the interaction of an appropriate T cell receptor (TCR) and MHC-presented peptide antigen (Signal #1) and a co-stimulatory signal (Signal #2).

*β<sub>2</sub>M*: beta-2 microglobulin

*Adapted from:* Kong HT, Restifo NP: Natural selection of tumor variants in the generation of “tumor escape” phenotypes. *Nature Immunol* 2002;3:999-1005.<sup>12</sup>